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FRAGILE X SYNDROME: THE ROLE OF MOLECULAR DIAGNOSIS AND SCREENING IN AN INTEGRATED APPROACH TO SERVICES

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Agence d'évaluation
des technologies
et des modes
d'intervention en santé

MISSION

To assist the Minister of Research, Science and Technology and the policymakers in Québec's health-care system, including the Ministère de la Santé et des Services sociaux, by means of health technology and intervention modality assessments, specifically, by assessing their efficacy, safety, costs and cost-effectiveness, and their ethical, social and economic implications.

To assist the Minister of Research, Science and Technology in developing and implementing scientific policy.

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FRAGILE X SYNDROME: THE ROLE OF MOLECULAR DIAGNOSIS AND SCREENING IN AN INTEGRATED APPROACH TO SERVICES

In 1996, the *Conseil d'évaluation des technologies de la santé* (*CETS*) started a project on the problems posed by the transfer of knowledge from medical genetics research to new clinical applications. The *Agence d'évaluation des technologies et des modes d'intervention en santé*, as *CETS* was renamed, with the support of a multidisciplinary committee, is thus examining technologies derived from molecular biology, which is making available an increased number of diagnostic and prenatal tests for many diseases.

CETS chose four diseases as a priority, namely, Duchenne and Becker muscular dystrophies, myotonic dystrophy, tyrosinemia and fragile X syndrome, its criteria being the seriousness and incidence of the disease, test availability and reliability, and the availability of preventive measures. The fourth and present report in this series concerns fragile X syndrome, the leading cause of mental retardation, after Down's syndrome. Its prevalence is at least 1 per 4,000 males and 1 per 8,000 females. Early clinical diagnosis of this disease is difficult.

This assessment summarizes the state of knowledge regarding the genotypic analysis of individuals with the syndrome and screening for asymptomatic carriers of a dynamic mutation on the FMR1 (fragile X mental retardation 1) gene. This report also discusses the usefulness of developing or maintaining such a service in Québec's health-care system and the management of affected individuals by medical, social and educational services, and explores the ethical issues involved.

In conclusion, the *Agency* believes that the conditions are in place for one or two laboratories meeting the necessary standards of quality to perform diagnostic tests for fragile X syndrome. Other implications regarding the clinical support and follow-up to be provided to the families concerned, the necessary medical, social and educational resources, research, and the organizational aspects of the services are discussed as well.

With this assessment, the *Agency* wishes to provide the best possible information to the policymakers in the many sectors concerned with this problem and its impact on affected individuals and families.

Renaldo N. Battista
President and Chief Executive Officer

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SUMMARY

Introduction

In 1996, the *Conseil d'évaluation des technologies de la santé* (*CETS*) started a project on the problems posed by the transfer of knowledge from medical genetics research to new clinical applications. The *Agence d'évaluation des technologies et des modes intervention en santé*, as *CETS* was renamed on June 28, 2000, with the support of a multidisciplinary committee, is examining technologies derived from molecular biology, which is making available an increased number of diagnostic and prenatal tests for many diseases.

CETS chose four diseases as a priority, namely, Duchenne and Becker muscular dystrophies, myotonic dystrophy, tyrosinemia and fragile X syndrome, its criteria being the seriousness and incidence of the disease, test availability and reliability, and the existence of preventive measures. Reports have already been published on the first three diseases. The present assessment therefore concludes this series on monogenic diseases.

In general, this report examines the state of knowledge regarding the genotypic analysis of fragile X individuals and healthy carrier screening, and explores the usefulness of developing such a service in our health-care system. Specifically, it first describes the disease, its management and its genetic aspects. It then investigates the impact on genetic counselling, examines the diagnostic indications and describes the molecular diagnostic protocols. Test performance, diagnostic strategies and especially strategies for screening for healthy carriers in the family and for screening pregnant women or women of child-bearing potential are discussed as well. Then, after describing the situation in Québec, the assessment devotes special attention to the many social and ethical issues surrounding the identification of the syndrome, to the professional interventions and to the different aspects of planning and organizing services. The report concludes by proposing specific approaches for adequately meeting the needs of affected individuals and families.

Overview of the syndrome

Fragile X syndrome is the second leading cause of mental retardation, after Down's syndrome. Fragile X syndrome is identified in about 2% of boys with mental retardation and in more than one third of families with a history of X-linked mental retardation. The prevalence is at least 1 in 4,000 males and 1 in 8,000 females.

This X-linked, dominant, monogenic disease exhibits an unusual mode of transmission, affecting both sexes, but with variable penetrance and expressivity. Most affected boys present with moderate to severe mental retardation, while only 55% of affected girls will have mental retardation, generally mild to moderate. Cognitive impairment can manifest as delayed language acquisition and developmental delay well before mental retardation is considered. The most typical physical signs (facial dysmorphia, macroorchidism) usually appear during adolescence, with behavioural problems, such as hyperactivity and attention disorders, appearing mainly during childhood and adolescence. The clinical picture therefore varies enormously, and no sign is pathognomonic. As a result, early clinical diagnosis is difficult, especially if there is no known family history of the syndrome.

While most of the needs generally result from mental retardation, language acquisition problems, learning disabilities, and behavioural and socialization problems create additional needs, which must be factored into the offer of services. The management of affected individuals requires regular medical follow-up, early intervention by functional reeducation professionals (speech therapists, occupational therapists, etc.), student services throughout the patient's schooling, and social integration and adaptation services. A concerted, multidisciplinary approach based on a needs assessment and individualized planning of services is best for optimizing the potential for these children's development and for promoting their autonomy and social integration. Additionally, the family requires information and support and should receive genetic counselling as soon as the diagnosis is made.

The contribution of genetics

In about 98% of cases, fragile X syndrome is linked to a "dynamic" mutation in the FMR1 (fragile X mental retardation 1) gene. The mutation is characterized by an expansion of variable size of a DNA sequence located in the gene's first exon and consisting of a variable number of CGG trinucleotide repeats. The alleles of the FMR1 gene are classified according to the number of repeats and the gene's methylation status. A normal allele contains between 6 and 54 triplets and is unmethylated; a premutation can contain between 55 and 200 triplets but is unmethylated; and a full mutation contains over 200 triplets and is methylated. The premutations and full mutations are unstable, their number of triplets usually increasing during mother-child transmission.

Methylation of the gene, which characterizes full mutations, seems to be what determines phenotypic expression, since it prevents the production of FMRP (fragile X mental retardation protein). Individuals who exhibit clinical signs of the syndrome therefore usually have a full mutation (or sometimes mosaics, allelic or methylation, with reduced FMRP production), whereas those who have a premutation are said to be asymptomatic carriers and are at risk for transmitting the syndrome to their offspring. The distinction between premutations and full mutations and the dynamic nature of the mutations explain why there are males and females who are obligate carriers but who are asymptomatic, the occurrence of the disease in families with no family history of mental retardation, and the increased penetrance in subsequent generations (the phenomenon of anticipation).

Molecular analysis of the FMR1 gene, which has been available as a clinical service since 1992, constitutes a substantial gain over the earlier cytogenetic analyses, which were prone to classification errors and unreliable in females. Molecular testing clearly establishes the diagnosis in symptomatic individuals, identifies individuals at risk for transmitting the syndrome and, by identifying the type of mutation, provides a more accurate estimate of the risk of transmitting the syndrome.

Developments in molecular genetics are thus an important contribution to genetic counselling, since they are making it possible to determine the genotypic status of an affected individual's relatives more accurately, reassuring some and permitting the others to make their reproductive decisions on the basis of a more accurate determination of the risks of transmitting the syndrome. However, certain limits in the current knowledge of genotype-phenotype correlations and of the meiotic stability of the alleles have an impact on genetic counselling and reproductive choices. The main problem concerns the decision facing couples when a prenatal diagnosis reveals a full mutation in a female fetus, since, in such cases, it is impossible to state with certainty if and to what extent there will be any intellectual impairment. All of these uncertainties and the resulting problems should be clearly explained to couples during genetic counselling so that they can base their decisions on sound, complete and up-to-date information.

Developmental delay and mental retardation of unknown etiology are formal diagnostic indications for fragile X testing, but they also warrant a broader workup with karyotyping, a genetic and neurological workup, and an assessment of the patient's cognitive and adaptive skills. Broadening the indications to language delay, attention disorders, autistic tendencies or learning disabilities in the absence of mental retardation would permit earlier diagnosis (so that the families could avail themselves of the necessary services sooner) and identification of a larger proportion of affected individuals (so that more families could obtain genetic counselling). However, relatively few studies are convincingly in support of systematically testing children with these isolated (i.e., in the absence of mental retardation) clinical signs. While the usefulness of the diagnostic test for these indications can be examined on a case-by-case basis in a clinical practice setting, the use of proactive diagnostic and carrier screening strategies founded on broadened indications should be based on more research and a more thorough assessment. Lastly, it now seems justified to screen for a premutation in prematurely menopausal women.

Genotypic analysis for fragile X syndrome entails the use of two techniques—Southern blot and PCR, which are performed sequentially. The reference method for diagnostic tests consists of the Southern method with double enzyme digestion, a modified method for identifying large expansions and determining the gene's methylation status, followed, if need be, by PCR in order to accurately determine the size of the normal and premutated alleles. This protocol permits genotypic diagnosis for most of the families concerned, since point mutations and deletions apparently account for at most 2% of the mutations associated with the syndrome. A number of researchers recommend protocols in which PCR is performed first, with selective confirmation by Southern blot. Such an approach would have the advantage of being faster and less expensive, feasible with specimens other than blood (e.g., a buccal smear) and better suited to large-scale screening. However, neither of these methods has been compared, in a rigorous and systematic fashion, with what is considered the reference method. Lastly, immunocytochemical analyses of FMRP expression are still in the research stage.

Instituting quality controls would be desirable, given the possible performance variability due to experimental conditions. Furthermore, the expertise required to interpret the results underscores the importance of close collaboration between research laboratories and clinical laboratories. Lastly, with regard to prenatal diagnosis, it is advisable to use both methods, Southern blot and PCR, routinely. Also, couples should be told that prenatal diagnosis, especially when performed by chorionic biopsy, can sometimes be inconclusive.

Situation in Quebec

Since no research project or program for identifying symptomatic individuals has been set up in Québec, it is difficult to accurately determine the number of affected people, especially since there is no register, association or specialized centre that can provide such data. According to a theoretical calculation based on the latest prevalence estimates, there were, in Québec in 2000, 250 affected children under the age of 15 years, for a total of 1,377 affected individuals. Upon comparing this figure with the approximate number of people in whom a diagnosis was made, it is seen that the syndrome is underdiagnosed, both in Québec and elsewhere. At the same time, a major study revealed that the prevalence of the premutation is 1 in 259 females in Québec.

Molecular tests for the FMR1 gene are performed by two laboratories. They have been available at the laboratory of CHUQ's Department of Biochemistry (Saint-François d'Assise Branch) since late 1991, and Hôpital Sainte-Justine's molecular biology laboratory developed a test in 1997. The reference method is currently used at the Saint-François d'Assise Branch, whereas at Hôpital Sainte-Justine's molecular biology laboratory, an alternative method in which PCR is performed first and whose analytical validation has yet to be documented in the literature, was used initially. However, in light of the preliminary results of a recent validation project carried out jointly with CHUQ's laboratory, those in charge of Hôpital Sainte-Justine's laboratory plan to modify their diagnostic protocol.

The number of test requests is gradually increasing, having been 700 to 800 annually for the past few years. It seems that an increasing number of requests are being made because of developmental delay and learning disabilities and that there is a trend toward earlier diagnosis. Given this trend toward using broader indications and the facts that the syndrome is underdiagnosed and that some families have not yet been identified, the demand is likely to remain stable or continue to grow in the next few years. The funds allocated for interhospital billing for genetic tests are one of the factors that could limit the demand for the tests and compromise equal access to them. Lastly, although the above-mentioned laboratories are currently able to meet the demand, their capacity should be reassessed in the event of a substantial increase in demand, which could happen if there is a practice change or if pilot projects are set up for the purpose of evaluating new diagnostic and screening strategies.

While it may be justified at this time to have, in Québec, two laboratories for performing these tests, considering the increasing demand for testing and the ordering recommendations issued by professional organizations, it would not be beneficial to increase the number of laboratories offering tests, given the expertise required to perform and interpret it and the importance of close collaboration between research laboratories and clinical laboratories. Furthermore, it would be beneficial for the diagnostic protocols to be harmonized and for a quality control system to be instituted for all molecular tests.

Upon examining the situation in Québec, it is seen that there is presently no specific medicosocial or educational management of affected individuals or families. With the movement to deinstitutionalize the mentally impaired and integrate them into schools and society, this management is provided both or alternatively by the medical community, CLSCs, schools and rehabilitation centres in conjunction with the family. Because of the involvement of all these sectors, with their respective responsibilities, and the progressive and multifaceted nature of fragile X syndrome, there are: 1) numerous points of entry and channels leading to a diagnosis; 2) nonstandardized approaches to the diagnostic workup; 3) different management modalities for reeducation and social integration; and 4) several types of educational paths.

This diversity of individual paths does not, as such, pose a problem as long as service accessibility, continuity and complementarity are ensured. These conditions can be fully realized only if the necessary services are available and effectively coordinated. Despite the efforts made in the health and social services system to improve organizational coordination and despite the options available through the development of individualized service plans for promoting service continuity and complementarity at the individual level, we must recognize that the obstacles to coordination contribute to hindering access to services and that optimal use of the resources is undermined because they are scattered. Furthermore, the availability of certain services has been reduced because of budget cuts over the last few years and the shortage of certain human resources in the health and social services and educational systems. Lastly, all of these problems are exacerbated by a lack of communication and collaboration between the medical, social and educational sectors.

The service accessibility, continuity and complementarity problems place an additional burden on affected individuals and their families. The parents, who already have to look after special-needs children on a daily basis, are often forced to undertake demanding administrative tasks and negotiate, in order to obtain services, with the various sectors responsible for them. As for affected children, the lack of timely services and the continuity problems, which disrupt the stability of their environment, could have a negative impact on their development.

Given the current deficiencies in the provision of services and given the responsibility of the promotors of a diagnostic and screening strategy, the usefulness of early diagnosis (for case-finding strategies) would hinge on improving, over the present situation, the accessibility, continuity and complementarity of the services provided to the families concerned. In this regard, it is seen that benefits could be achieved as long as a substantial effort to inform professionals enables them to use the diagnosis as a service planning and coordination tool.

While there may not be any recognized direct therapeutic benefit for affected individuals, early diagnosis would unquestionably be beneficial in terms of defining their needs and those of their families. Confirming the diagnosis in a symptomatic individual could thus contribute to better planning of the necessary care and services. Even if this contribution is difficult to assess, it would be of the utmost importance to design the offer of testing in tandem with better coordination of efforts to improve the referral of affected individuals and their families to the available services, to improve timely access to the necessary services and to adapt the available services to the specific needs of affected individuals and their families.

Diagnostic and Screening Strategies

Depending on whether one diagnoses and screens for affected individuals (current clinical diagnostic practice; prenatal diagnosis; diagnosis and proactive screening in high-risk populations; neonatal screening) or screens for carriers at risk for transmitting the syndrome (cascade screening, screening of women with a history of mental retardation or other signs; screening of pregnant women with no particular family history; preconceptional screening of women of child-bearing potential), the objectives are not the same. With regard to diagnosing and screening for affected individuals, the impact usually anticipated when making an accurate diagnosis is appropriate medical, psychosocial and educational management of the affected individual. As for carrier screening, its purpose is to identify individuals at risk for transmitting the syndrome to their offspring and, through genetic counselling, to enable them to make informed reproductive choices. Usually, cascade screening is performed after an individual is diagnosed with the syndrome.

To evaluate screening strategies, one assesses the utility, feasibility and acceptability of the various proposable strategies. To do this, accumulated international experience should be applied to the local context, which partly determines their feasibility and acceptability, as well as their utility. In this regard, too often we have to rely on incomplete data concerning the utility and acceptability of strategies for which international experience is limited.

A number of arguments can be presented to demonstrate the usefulness of screening low-risk populations. The syndrome is underdiagnosed; about one third of cases of the syndrome occur in families with no family history of mental retardation; the prevalence of premutations is dation; the prevalence of premutations is high; and the proportion of carriers that can be identified by screening high-risk individuals followed by cascade screening would be significantly lower than the results anticipated for more systematic screening of low-risk populations. However, neonatal screening cannot be recommended at this time because of the problems involved in identifying premutation carriers and because early management does not confer any benefit. Also, preconceptional screening and the systematic screening of pregnant women with no particular family history are subject to the limits of our prognostic capabilities. These weaknesses basically concern the risk of a maternal premutation expanding into a full mutation in her children where there is no family history and predicting cognitive impairment in female fetuses carrying a full mutation, two essential elements for determining the risk of transmitting the syndrome to the offspring. Since screening low-risk women is recognized as potentially having some usefulness, despite what little experience has accumulated at the international level, it is to be hoped that the pilot projects currently underway in various countries are adequately examining the benefits, risks and main issues that determine the acceptability of these approaches.

As for proactive high-risk-population screening strategies, there is vast international experience suggesting that the existing programs are generally well accepted and that they meet a need on the part of families, if followed by cascade screening. However, this approach has certain limitations that are now clearly known: these programs end up achieving a decreasing case-finding yield; the screening of the families is often incomplete; and carriers with no family history cannot avail themselves of this service. Furthermore, most studies have concerned themselves with a limited number of indications, mainly mental retardation, and therefore do not address the additional objective—now a given—of identifying affected individuals as early as possible. Achieving this objective would require a broadening of the diagnostic indications and the concurrent use of other selection criteria, two facettes of screening on which there is precisely no consensus in the literature. Also, the feasibility of this strategy clearly depends on the regional organization of services and especially on the centralization of the educational and specialized services provided to the clientele of interest. Yet, it seems that, in Québec, given the extent of the movement toward social and school integration, the management of developmentally delayed, mentally retarded and learning-disabled children varies enormously and is very decentralized, both from a medical, social and educational standpoint. Lastly, a selective, proactive approach in Québec has not yet achieved social acceptability because the population and many health professionals are ill-informed about the syndrome.

Consequently, no proactive high-risk-population screening strategy or no low-risk-population screening strategy can be recommended at this time due to the facts that such strategies raise numerous ethical issues which need to be debated and that a number of scientific problems first need to be resolved. Furthermore, there would need to be validated tests suited for wide-scale screening. The methods and knowledge in this area are evolving at an extremely rapid pace, with the result that, in the future, the situation will have to be reassessed in light of the new developments.

For the time being, we will therefore have to rely more heavily on an improvement in the current practice of diagnosing affected individuals (clinical practice meeting the demand of families of symptomatic individuals) and of screening for people at risk for transmitting the syndrome to their offspring (cascade screening and prenatal diagnosis if there is a family history of the syndrome; screening of pregnant women with a family history of mental retardation if the diagnosis cannot be made beforehand in a symptomatic relative). In this regard, the deficiencies found upon examining the situation in Québec are of several types. The variability and insidious occurrence of the symptoms and the relative lack of knowledge of this clinical entity by first-line health professionals, the numerous points of entry into the system, the lack of collaboration between the health-care system and the school and preschool system, and the absence of a standardized workup for developmental delay and mental retardation lead to highly variable recruitment channels and unplanned prescreening and result in the fragile X syndrome remaining underdiagnosed. In addition, the "prediagnostic" path is described by parents as being difficult and painful, and the psychosocial support system after the diagnosis is communicated, timely access to genetic counselling, and referrals to the necessary support and integration services vary enormously. Lastly, the mode of service organization not only has an impact on the recruitment channels leading to a diagnosis, but also on the management of affected individuals and their families.

Improvement to the current practice could therefore be considered at several levels: that of professional practice and that of the organization of services, together with broader dissemination of information. From the standpoint of professional practices, the development and dissemination of a standardized workup for developmental delay and mental retardation would have the advantage of promoting greater diagnostic accuracy, which would be of benefit to all the children concerned. From an organizational standpoint, the problem of intersectorial coordination is the most obvious one. Any effort to make improvements should essentially be aimed at promoting the integration of the diagnostic and medical follow-up services, the cognitive and functional assessment, and the offer of reeducation and integration services. Such an objective could, for example, be achieved either by setting up a referral network or by creating truly multidisciplinary centres dedicated to mental retardation, but also to precursor signs, such as developmental delay or language acquisition delay. Any steps in this direction will mean greater responsibility on the part of the professionals and organizations involved and substantial cooperation.

Ethical and Social Aspects

Fragile X syndrome diagnosis and screening in affected families, the situations in which the tests are ordered, and the organization of the services relating thereto have certain ethical and social implications.

In the identification of fragile X syndrome, the actual diagnostic workup, the communicating of the results, and the follow-up that is subsequently proposed raise certain ethical issues. The impact of a diagnosis of fragile X syndrome varies according to the setting and the situation in which the test is ordered and according to the individual or individuals concerned. The impact of identifying the syndrome, providing information to the families, the use of prenatal diagnosis, attitudes toward aborting an affected fetus and attitudes toward screening and communicating the fact that a child is a carrier are discussed in this report. The problems of stigmatization and discrimination, which are most often brought up in the context of using genetic diagnosis for nonmedical purposes, can occur more acutely in the context of screening programs, like those carried out elsewhere in schools for fragile X syndrome. The sources of stigmatization are numerous. They can be the school, the community or those in charge of screening.

The quality of fragile X syndrome diagnostic and screening services depends on the test's efficacy, on the quality of the laboratory services and on the provision of clinical services, which require a sufficient number of competent personnel. A genetic test should be performed within the context of a medical procedure, with the practice standards and the protection that this context provides. For it to be judiciously incorporated into medical practice, the offer of testing should be accompanied by genetic counselling adapted to the particular characteristics of the syndrome so that complete and adequate information is provided and in order to give the psychosocial support required when providing this information. Respect for autonomy, privacy and confidentiality, ethical principles that underlie the standards of medical practice, receive an interpretation specific to the context of genetics. These practice standards, which medical genetics has adopted, should be in effect when other medical specialties are involved in the use of the tests and in providing genetic information, as is often the case for fragile X syndrome. Additionally, the possibility of commercializing the tests raises several questions relating to the assessment of their value, their interpretation, quality control and the concurrent provision of appropriate medical follow-up and genetic counselling.

Instituting a diagnostic and screening service is also a concern of the public authorities. From the standpoint of health care, the public authorities' responsibility extends to the offer of services considered necessary, the allocation of resources to different services, the organization of clinical and laboratory services, and quality assurance. As for fragile X syndrome, the numerous organizations and people involved in the offer of services, the lack of collaboration between the health-care system and the educational system, the lack of availability for ensuring efficient coordination of the services, and the numerous cuts to specialized services are forcing parents to spend time and money to obtain access to services. In short, the current context of sharing responsibilities with regard to organizing services has major repercussions on the recruitment channels leading to the demand for diagnostic tests and on the accessibility to these services by individuals with the syndrome and their families.

The social and ethical aspects of identifying fragile X syndrome underscore the need for clear guidelines for the diagnostic and screening services, this to ensure their potential benefits and minimize the prejudice that can accompany the institution of such services. Genetic professionals, who have made a significant contribution to the emergence of the current standards in medical genetics, and the health and social services professionals involved in the diagnostic workup and in providing functional reeducation and social integration services, who are in direct contact with the families, have a responsibility in this regard. Secondly, the legislature and the public authorities should put in place the necessary conditions for guaranteeing the quality and accessibility of the services offered and protect the individuals from any prejudice. It goes without saying that these services should be dispensed in accordance with the basic ethical principles of respect for a person's dignity, autonomy and privacy, and with the values that are part of the health-care system, such as fairness.

Conclusion

Consequently, the Agency believes that:

1. The necessary medical, social and educational resources should be available to meet the needs of affected families in a timely and appropriate fashion.

2. The different players in the health and social services and educational systems should examine the possible ways of improving early identification and the diagnostic workup of children with signs consistent with fragile X syndrome, devoting special attention to the services available for developmentally delayed children.

3. One or two laboratories should be available to perform, for all of Québec, the molecular tests for fragile X syndrome in the following situations:
    - The molecular diagnosis of fragile X syndrome in a symptomatic individual with either an indication recognized in the medical association guidelines or signs consistent or associated with the syndrome, in the opinion of the ordering physician.
    - Cascade screening of an affected individual's relatives.
    - Confirmation of carrier status in a pregnant woman with a family history of signs associated with the syndrome.
    - Prenatal diagnosis, if the mother is a carrier of a premutation or a full mutation.

4. All laboratory services should be subjected to quality control.

5. The different players in the health and social services system, the educational system and the job sector should improve intersectorial collaboration at the regional level in order to improve the coordination and continuity of the services available to affected individuals and their families.

6. The issues of the accessibility, continuity and complementarity of services for fragile X syndrome reflect, in part, an organizational problem that also affects children with developmental delays of other etiologies and their families, with the result that the required efforts should be part of a coherent approach that will benefit all these families.

7. The public authorities should give preference to a mode of service organization that promotes the respect of individuals, ensures equal access to services in all regions and prevents discrimination, especially in the area of insurance.

8. Research should continue, here and elsewhere, to better document the following:
    - The epidemiology of the syndrome in the general population.
    - The risk of hereditary transmission of the syndrome.
    - Phenotype prediction.
    - The development of genetic tests better suited to wide-scale use.
    - The psychosocial impact of diagnosing, screening and genetic counselling.

9. It would be essential to evaluate, by means of pilot projects, any high- or low-risk-population diagnostic and screening strategy whose implementation is being considered, on the basis of the following criteria:
    - Its technical, organizational and economic feasibility.
    - Its efficacy in terms of the number of individuals or couples who have received genetic counselling and follow-up that meet their needs.
    - Its usefulness in terms of the services that are already available.
    - Its ethical and social acceptability.

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FOREWORD

The problems posed by genetic medicine are many and complex, especially those that concern the transfer of knowledge from research to new clinical applications. To examine these issues, the *Agence d'évaluation des technologies et des modes d'intervention en santé* formed a research team and a multidisciplinary committee that could enlighten it about the major problems posed by genetic technology in the context of health-care planning. The task of the committee, whose role is basically to provide information to the research team and guide its work, was to help determine which aspects of these developments should be examined, which criteria should be used to evaluate genetic screening programs and which research strategies would be appropriate.

Specifically, we took an interest in genetic technologies derived from molecular biology, which has made an increased number of diagnostic and prenatal tests available these past few years. These tests, which are first available in research projects, find their clinical applications relatively soon. The transfers sometimes constitute a grey area that escapes any proactive (*a priori*) evaluation, a desirable exercise ensuring successful implementation within the overall framework of the health-care system.

Of the different diseases for which molecular genetics is changing medical practice, we previously chose and evaluated Duchenne and Becker muscular dystrophies, myotonic dystrophy and hereditary tyrosinemia type I. Using the priority criteria of the seriousness of the disease, its incidence, test reliability and the availability of preventive measures, we identified a fourth monogenetic disease—fragile X syndrome.

This disease is the most frequent cause of hereditary mental retardation and the second leading cause of mental retardation, after Down's syndrome. Fragile X syndrome has been identified in more than one third of families in which X-linked mental retardation is found. Mental retardation is the most prominent characteristic of the syndrome, but the manifestations of this disease vary from one individual to another and appear gradually with age. Since no sign is pathognomonic and since its most typical signs (facial dysmorphia and macroorchidism) appear late, early clinical diagnosis is difficult. This monogenetic disease exhibits an unusual mode of transmission. It is X-linked but dominant, with variable penetrance. It affects both sexes, but boys are generally affected more severely than girls. Both males and females can be asymptomatic carriers of the mutated gene and are therefore at risk for transmitting the syndrome to their offspring.

The identification of the gene responsible for fragile X syndrome, the FMR1 gene, in 1991 and the characterization of the type of mutation associated with it constitute a major advance in understanding the hereditary transmission of this disease. Furthermore, a molecular analysis-type test has been developed that clearly establishes the diagnosis in symptomatic individuals, identifies individuals at risk for transmitting the syndrome and, by determining the type of mutation, permits a more accurate estimate of the risk of transmission of the syndrome in an affected individual's relatives. These developments have contributed significantly to the genetic counselling of affected families, reassuring some and permitting the others to make their reproductive decisions with full knowledge of the facts.

This monograph sheds light on the state of knowledge regarding the genotypic analysis of affected individuals and on healthy carrier screening, and examines the usefulness of developing such a service in our health-care system. In one section, we provide an overview of the disease and explain its management and genetic aspects. In another, we explore the impact on genetic counselling, examine the diagnostic indications and describe the molecular diagnostic protocols. Diagnostic strategies and especially strategies for identifying healthy carriers in the family and for screening pregnant women or women of child-bearing potential are discussed. The other sections concern test performance, the situation in Québec, the many social and ethical issues surrounding the identification of the syndrome, the professional interventions, and the planning and organization of services. More in-depth discussions, mainly of different technical aspects, and of the organization of reeducation, rehabilitation and educational services are provided in the appendices.

The bulk of this report is based on a thorough MEDLINE literature search from 1991 on, which was complemented by hand-searching the references cited. As for the examination of the situation in Québec, it is based on a study of articles and research reports on the living conditions of the mentally impaired, the policies concerning the services intended for them, publications by parents' associations and interviews with key informers.

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GLOSSARY

Alleles: Alternate forms of a given gene that differ in their nucleotide sequence.

Allelic mosaic: A type of mosaic resulting from somatic instability. It is characterized by the presence of premutations in certain cell lines and full mutations in others.

Amplimer: The product of *in vitro* PCR amplification.

Analytical sensitivity of molecular genetic tests: The proportion of individuals with the genotype of interest (the mutations detectable by the test) in whom the test will be positive.

Analytical specificity of molecular genetic tests: The proportion of individuals who do not have the genotype of interest (the mutations detectable by the test) and in whom the test will be negative.

Anticipation (phenomenon of): In the case of fragile X syndrome, the phenomenon of anticipation is characterized by increased penetrance in successive generations (a greater proportion of symptomatic individuals), not by increased expressivity (an increase in symptom severity), as is the case in other diseases caused by dynamic mutations.

Autosome: Any chromosome other than a sex chromosome (22 pairs of autosomes).

Chromosome: The genetic material, which is contained in the cell nucleus and consists of DNA, is divided into 46 chromosomes. There are 22 pairs of autosomes and two sex chromosomes.

Clinical sensitivity of molecular genetic tests: The proportion of individuals with the phenotype of the disease (or who will develop this phenotype) in whom the test will be positive. A more theoretical interpretation of this definition is sometimes used by considering only the proportion of individuals with the phenotype of the disease (or who will develop this phenotype) in whom the mutations targetted by the test are present. In a way, this definition represents the upper limit of clinical sensitivity when the analytical sensitivity is 100%.

Clinical specificity of molecular genetic tests: The proportion of individuals who do not have the phenotype of the disease (and who will not develop this phenotype) and in whom the test will be negative.

Deletion: The loss of one or more consecutive base pairs without a break in the continuity of the DNA molecule.

DNA (deoxyribonucleic acid): The genetic material contained in the chromosomes and mitochondria. It consists of two chains made up of substances called *nucleotides* and coiled into a double helix. There are four types of nucleotides, and each can pair only with one other, the sequences on each strand being complementary to those on the other. The genetic information is coded in the form of a sequence of codons or trinucleotides that correspond to an amino acid or to a translational stop signal.

DNA polymerase: An enzyme capable of synthesizing *in vitro*, by elongation of a primer, a DNA strand whose sequence is complementary to the DNA strand that serves as a template and to which the primer has annealed.

Dominant: An allele is dominant when it is expressed even in the heterozygous state, i.e., when it is present on only one of the two homologous chromosomes. The carrier of a dominant disorder has inherited the mutation from one of his or her parents, unless there was a new mutation. Each child of an affected person will inherit either a normal gene or an abnormal one. The probability that he or she will be affected is therefore 1 in 2. Cf. *recessive*.

Exon: A gene sequence whose transcript persists in the final messenger RNA and which can therefore be translated into a polypeptide chain. Each gene contains several noncontiguous exons, which are separated by introns.

Expansion: The abnormal repeating of a DNA sequence, ranging in length from two or three nucleotides (triplets) to entire genes.

Expressivity: The degree of intensity of the morbid manifestations of a hereditary trait.

Gene: A physical and functional unit of heredity, being a sequence of nucleotides situated at a specific locus on a given chromosome and performing a specific function.

Gene therapy: The modification of the genetic composition of a cell of an individual that has a defective gene. It is called *somatic gene therapy* if it applies to cells of the body other than germ cells (eggs and spermatozoa) and *germ-line gene therapy* if it applies to germ cells.

Genetic linkage: The cosegregation of two or more alleles over the generations because of the physical proximity of their loci on the genome.

Genetic marker: Any variation in the DNA sequence creating different alleles at a given locus and used to identify this locus.

Genetic polymorphism: The occurrence, in a population, of at least two allelic variants at a given gene locus. They can be investigated by DNA analysis (genotypic polymorphism) or protein product analysis (phenotypic polymorphism).

Genetics: The study of the structure, regulation, expression, transmission and frequency of genes, and of the pathologies associated with structural defects in genes.

Genetic screening: The use of tests to obtain genetic information about individuals belonging to groups in which there is a relatively high risk of inheriting a given trait or disease.

Genetic test: A test for detecting a mutation, a defective gene, an abnormal protein, a chromosomal abnormality or the presence of a genetic marker near or in a gene.

Genome: The genetic material contained in the complete set of chromosomes of an individual's cells. The human genome contains about 100,000 genes.

Genotype: An individual's genetic makeup, as contrasted with his or her phenotype.

Heterozygosity: A genotypic situation in which two homologous loci in a given chromosome pair each carry a different allele. In the case of the X chromosome, only females can be heterozygous.

Homozygosity: The presence of the same allele on both chromosomes in a given chromosome pair. By extension, is said of the genotype of individuals who have inherited a double dose of an abnormal allele, whether the mutated version is the same or different on each chromosome.

Intron: A DNA sequence transcribed and subsequently eliminated by splicing during RNA processing.

***In vitro* selective amplification:** See *PCR*.

Kilobase (kb): In the case of DNA, 1,000 base pairs (bp); in the case of RNA, 1,000 bases.

Locus: The position of a DNA segment on a chromosome. It is defined by its information content (gene) or its sequence, whether the latter is polymorphic or not.

Meiosis: The two cell divisions in gametogenesis, during which the number of chromosomes is reduced by one half, ending in the formation of four haploid cells (with 23 chromosomes instead of 46). See *mitosis*.

Mendelian trait or disease: A characteristic or disease due to a single gene transmitted by a simple pattern of inheritance (autosomal dominant, autosomal recessive, X-linked).

Methylation mosaic: A type of mosaic characterized by variation in the degree of methylation of a full mutation from one cell line to another.

Mitosis: Cell division involving somatic cells as opposed to germ cells. See *meiosis*.

Molecular hybridization: Base pairing (G-C and A-T) of two complementary nucleotide sequences. The duplex thus formed can be of the type DNA/DNA, DNA/RNA or RNA/RNA.

Monogenetic: Said of a hereditary disease due to a mutation in a single gene. The lesion can be monoallelic (drepanocytosis) or multiallelic (β-thalassemia).

Mosaic: Said of an individual or a tissue whose cells do not have the same genetic makeup. A germ-line mosaic implies that only the gametes (and their precursors) have two or more genetically different cell lines. A somatic mosaic implies that other body tissues consist of at least two genetically different cell lines.

Mutation: Any change occurring in the DNA sequence that can result in pathological manifestations. If the change involves only one base, one speaks of a point mutation. If a mutation occurs in a germ cell, it can be passed onto subsequent generations. A gene that has undergone mutation is called a *mutant*. The term *mutagenic* refers to a substance that can cause a mutation.

Nuclear family: A family nucleus consisting of parents and their children (as opposed to the extended family, which can include grandparents and more-distant relatives).

Nucleotides: Basic units of DNA and RNA structure. They consist of a phosphorylated sugar (ribose or deoxyribose) bound to a base. Two purine bases, adenine (A) and guanine (G), and two pyrimidine bases, cytosine (C) and thymine (T), are constituents of DNA. In RNA, thymine is replaced by uracil (U). Each strand of DNA consists of a sequence of nucleotides, whose bases can pair two by two (adenosine with thymine and guanine with cytosine) to form the DNA double helix.

PCR (polymerase chain reaction): The selective amplification of a sequence of double-stranded DNA carried out *in vitro* by the iterative extension of two primers, one on either side of the region of interest, using a DNA polymerase. Amplification is effected by repeated cycles of denaturation, annealing and extension, which result in the logarithmic replication of each strand.

Penetrance: The percentage of individuals that have a specific genotype and in whom the phenotype associated with this genotype is expressed.

Phenotype: The outward manifestation of the makeup of the genome in the form of a morphological trait, clinical syndrome or physiological characteristics, such as a qualitative or quantitative variation in the final product expressed by a gene (protein or metabolites).

Premutation: A type of allele encountered in dynamic mutations. It contains a higher-than-normal number of trinucleotide repeats and exhibits meiotic instability but is usually not accompanied by clinical manifestations.

Prevalence: The ratio of the number of individuals with a disease over the number of individuals in a given population at a given point in time. Birth prevalence is the number of affected newborns over the number of births during a given period of time.

Primer: A DNA sequence of approximately 15 to 30 nucleotides (oligonucleotide) serving as an anchor point and starting point for the replication, by DNA polymerase, of a specific DNA sequence during PCR.

Probe: A nucleic acid sequence, usually at least 15 nucleotides in length, homologous to a DNA or an RNA sequence, to which it anneals in a stable and specific manner by reassociation between complementary nucleotides.

Recessive: Said of an allele or a mutation that does not affect the phenotype in the heterozygous state. Refers to a mutated gene that will result in an abnormal phenotype only if it is present on both homologous chromosomes. If the mutated gene is present on only one of the two chromosomes of a pair, the individual will be a carrier of the disease but will not be affected (healthy carrier). However, in a male, the presence of a single mutated gene on the X chromosome will result in its phenotypic expression. Cf. *dominant*.

Restriction enzymes: Bacterial endonucleases that specifically cleave the two DNA strands at a particular DNA sequence (4 to 8 nucleotides), referred to as a *restriction site*.

Restriction polymorphism: A variation in the nucleotide sequence that modifies the action of a restriction enzyme and therefore the size of the restriction fragments.

Restriction site: The double-stranded DNA sequence specifically recognized and cleaved by a given restriction enzyme.

Sex chromosomes: The X and Y chromosomes, which determine sex. The presence of an X and a Y determines masculine sex, that of two X's female sex.

References: Bridge, 1994; Forestier and Schorderet, 1997; Royal Commission on New Reproductive Technologies, 1993; Kaplan and Delpech, 1994; Rousseau, 1996.

1. FRAGILE X SYNDROME

1.1 OVERVIEW OF THE DISEASE

In the early 1940s, Martin and Bell (1943) described a familial form of mental retardation, whose hereditary transmission seemed to be X-linked, since it basically affected males. It was only 26 years later that Lubs (1969) described the existence of a fragile site, identifiable by cytogenetic analysis, on the long arm of the X chromosome. It took several more years for confirmation of these observations and a better description of the syndrome's clinical and epidemiological characteristics.

Besides mental retardation of variable severity, individuals with Martin-Bell syndrome, or fragile X syndrome, may present with mild facial dysmorphia, macroorchidism and behavioural problems. The clinical signs not only vary, but they also appear in a very insidious manner with age. Furthermore, no sign is pathognomonic, with the result that the diagnosis cannot be made on the basis of the clinical findings alone.

The experimental conditions necessary for identifying the fragile site (now called the FRAXA fragile site) were not defined until 1977, by Sutherland (1977), which made it possible to conduct more in-depth studies of the syndrome. Cytogenetic analysis proved useful for detecting the syndrome in affected individuals but not for identifying asymptomatic carriers.

Despite these diagnostic problems, fragile X syndrome was recognized as one of the most frequent causes of mental retardation, after trisomy 21 (Down's syndrome). It is believed to be implicated in 30 to 40% of families with X-linked mental retardation (Sherman, 1996; Curry et al., 1997; King et al., 1997; Feldman, 1996).

In the mid-1980s, the syndrome's simple mendelian mode of hereditary transmission was questioned, given the description of several unusual characteristics for X-linked transmission[1]. First of all, it was observed that up to 35% of female carriers show clinical involvement, which is usually less severe than that in males, and that 20% of males who are obligate carriers, based on pedigree analyses, are asymptomatic. Additionally, cytogenetic analysis does not reveal a fragile site in these individuals, who are referred to as "normal transmitting males" (NTMs), just like in more than 50% of females who are obligate carriers. It was concluded that transmission of the syndrome was X-linked but dominant, with incomplete penetrance (estimated at 80% in males and 35% in females) (Sherman et al., 1984; 1985). Lastly, the incidence of the syndrome varies from one generation to the next. On the one hand, the phenomenon of anticipation occurs, i.e., an increased concentration of symptomatic individuals in the more recent generations. On the other, the probability of observing the disease is greater in an affected individual's siblings than in the siblings of a normal transmitting male, a phenomenon known as "Sherman's paradox" (Sherman et al., 1984, 1985).

Thanks to advances in molecular genetics, these enigmas are being resolved. The discovery, in 1991, of the gene (Verkerk et al., 1991), referred to as the FMR1 (fragile X mental retardation 1) gene, and the description of dynamic mutations (Fu et al., 1991; Oberlé et al., 1991; Kremer et al., 1991) paved the way to a better understanding of the risk of transmission in affected families and therefore to more informed genetic counselling. Indeed, these discoveries led to the development of molecular tests that can be used not only to diagnose affected individuals, but also to determine which at-risk individuals in the families concerned are asymptomatic carriers, which is now the main approach to preventing the disease.

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[1] The X-linked mode of hereditary transmission is suspected in the pedigree analysis if there are at least two affected males, either over two generations of the family or in two related sibships on the maternal side, and if there is no father-son transmission (Feldman, 1996).

However, the role of the gene product FMRP (fragile X mental retardation protein) has still not been completely elucidated, and the pathophysiologic bases of the syndrome are still unknown, with the result that there is no curative treatment (Kooy et al., 2000). Management is basically of the symptomatic type and requires, depending on the affected individual's age and the extent of his or her mental retardation, the collaboration of several health and social services, education and occupational professionals.

1.2 EPIDEMIOLOGY

The consensus in the most recent literature seems to be that the prevalence of fragile X syndrome is approximately 1 per 4,000 males and 1 per 8,000 females (Murray et al., 1997), while up until 1996, the most-cited prevalence figures were about 1 per 1,000 to 2,000 males and 1 per 2,000 to 4,000 females (Sherman, 1996; Turner et al., 1986; Webb and Bundey, 1991; Nussbaum and Ledbetter, 1995; Rousseau et al., 1995). Actually, no population study has been carried out on a representative sample large enough to accurately determine the syndrome's prevalence.

In fact, the prevalence estimates have been extrapolated from the number of subjects identified in high-risk populations. And the target populations varied substantially from study to study, not only in terms of the indications for testing (mental retardation, developmental delay, learning disabilities, etc.), but also in terms of the selection criteria (criteria of having been institutionalized, classification of children with special learning needs, exclusion or inclusion of cases of mental retardation of known etiology, use of additional criteria for clinical prescreening, etc.), with the result that comparing sometimes very divergent results is difficult[2]. Furthermore, the method of calculation for extrapolating the prevalence and the definition of the reference population for calculating the denominator are not always comparable.

Lastly, the first studies were based on the use of cytogenetic tests for diagnostic purposes. This method resulted in an overestimate of the prevalence because, among other things, of the existence of several fragile sites on the long arm of the X chromosome near the FRAXA site (false positives). Two authors published a comparison of the results obtained by cytogenetics (Webb et al., 1986; Turner et al., 1986) and those obtained by molecular analysis in the individuals who were initially identified (Morton et al., 1997; Turner et al., 1996, 1997). Consequently, they adjusted their initial prevalence estimates downward[3]. Since these estimates are consistent with those in a subsequent study in which molecular analysis was used (Murray et al., 1996), Murray et al. (1997) concluded that the prevalence of 2.5 per 10,000 males (or 1 per 4,000 males) is presently the best available estimate[4], although it should be considered a lower limit because of the method by which subjects were recruited for these studies. Since these studies targetted school-age children, a certain number of unsymptomatic children or children who did not attend the institutions of interest may have escaped the diagnostic process. Furthermore, as a result of confirming, by molecular analysis, only those subjects in whom the cytogenetic test was positive, the false negatives with cytogenetic analysis cannot be verified in any way (Murray et al., 1996). The prevalence in females has been estimated from the prevalence in males and is believed to be about one half thereof. The syndrome is therefore expressed in approximately 1 per 8,000 females (Murray et al., 1997).

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[2] For summary tables and/or a more detailed discussion of the studies, see Sherman (1996), Murray et al. (1997), Hagerman et al. (1988), Fisch et al. (1988) and O'Dwyer et al. (1997).

[3] Even if these studies are some of the most exhaustive ones, the adjustments that were made involved a small number of cases, with the result that the margin of error for these revised estimates may, perhaps, not be insignificant. For example, a large study carried out later with molecular tests reported a prevalence of 1 per 6,045 males, with a 95% confidence interval of 1 per 3,851 to 1 per 9,981 (de Vries et al., 1997).

[4] Other studies report estimates pointing to a lower prevalence: Tranebjaerg et al. (1994), Mazurczak et al. (1996), de Vries et al. (1997), Millan et al. (1999) and Youings et al. (2000).

Based on a literature review, Murray et al. (1997) estimate that approximately 6% of males institutionalized for mental retardation have fragile X syndrome[5], whereas the studies that they identified, which involved noninstitutionalized individuals, report a prevalence of 2 to 6%.

1.3 CLINICAL DESCRIPTION[6]

The clinical signs of fragile X syndrome appear gradually during childhood. They vary enormously from one individual to another and are generally more severe in males than in females. The syndrome does not seem to affect life expectancy, and no particular cause of death is reported for affected individuals.

The clinical picture is dominated by mental retardation (MR), which is generally moderate to severe in boys and often less severe in girls. Cognitive impairments can manifest as language acquisition delay and developmental delay well before mental retardation can be confirmed. The physical signs, including facial dysmorphia, are initially discreet and become more obvious around adolescence, which often delays the diagnosis. Behavioural problems, on the other hand, will occur mainly in childhood and adolescence.

The clinical description below concerns the signs and symptoms observed when the clinical expression of the syndrome is complete. However, it should be borne in mind that it is a syndrome, in other words, a constellation of signs and symptoms whose combination may point to a diagnosis, but which are rarely all present and of which none is mandatory[7]. In the section on phenotype/genotype correlations (Section 2.4), we discuss the range of clinical manifestations and the link between the syndrome's clinical expression and the exact nature of the mutations.

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[5] Unweighted mean derived from 10 studies essentially based on cytogenetic analysis, with results ranging from about 2 to 16%.

[6] A number of articles provide a detailed description of the clinical and behavioural signs of the syndrome: Hagerman, Bennetto and Pennington (1996), Abbeduto and Hagerman (1997), de Vries et al. (1998b) and Hagerman and Lampe (1999).

[7] According to a multicentre study, 58% of affected females and 6% of affected males (full mutation) do not exhibit any dysmorphic signs (Rousseau, 1994).

Table 1.1: Proportion of affected males and females with various degrees of mental retardation

Mental retardation	Males (n=243)	Females (n=161)
None	0%	41.0%
Borderline	2.9%	20.5%
Mild	6.5%	17.4%
Moderate	45.7%	13.0%
Severe	28.4%	4.4%
Profound	16.5%	3.7%

Source: Rousseau (1994).

1.3.1 Cognitive impairments

Almost all adult males with the syndrome have mental retardation[8], which, in most cases, is moderate to severe, their mean IQ being around 40, while women are less frequently and usually less severely affected. Table 1.1 summarizes the results of a multicentre study involving a large number of affected individuals[9]. However, in that study, mental retardation was assessed on the basis of clinical judgment, not on an IQ determination by means of psychometric testing.

In a large percentage of boys, a decline in IQ with age[10] has been described; it occurs toward the end of childhood and in adolescence[11] (Wright-Talamante et al., 1996; Fisch et al., 1996b). Detailed neuropsychological studies seem to indicate more pronounced impairment of certain abilities (arithmetic, spatial orientation and visual-motor coordination, integrated sequential activities, short-term memory), while other cognitive functions are more or less spared (reading, long-term verbal memory) (Bennetto and Pennington, 1996; Mazzocco, 2000).

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[8] The conventional distinction between the various degrees of mental retardation based on IQ measurements (mild for an IQ of 50-55 to 70; moderate for an IQ of 35-40 to 50-55; severe for an IQ of 20-25 to 35-40; and profound for an IQ under 20-25) is still used and is still useful in certain areas of research. However, there is a trend toward it being replaced by a finer analysis of each individual's strengths and weakness with regard to his or her adaptive skills, which better reflect the disabilities resulting from his or her mental retardation and which permit a better assessment of the resulting handicap in his or her environment and the support needed for dealing with it. The definition of mental retardation proposed by the American Association on Mental Retardation in 1992 (1992) is as follows: "Mental retardation refers to substantial limitations in present functioning. It is characterized by significantly subaverage intellectual functioning, existing concurrently with related limitations in two or more of the following applicable adaptive skill areas: Communication, self-care, home living, social skills, community use, self-direction, health and safety, functional academics, leisure and work. Mental retardation manifests before age 18." Approximately the same definition is given in the diagnostic manual DSM-III-R.

[9] The genotypic status (full mutation) was confirmed by DNA analysis.

[10] In children with Down's syndrome, a gradual decline in IQ was observed as well, but this is not necessarily the case in all mentally retarded children.

[11] Other studies seem to indicate that it could be the same for adaptive skills (Dykens, 1993; Dykens et al., 1995) and for girls (Fisch et al., 1997).

Table 1.2: Proportion of affected males with various physical signs, by age

Physical signs	< 12 years	≥ 12 years
Long face	64%	80%
Prominent ears	78%	66%
High-arched palate	51%	63%
Excessive ligament laxity	81%	49%
Macroorchidism	54%	92%

Source: According to a study by Merenstein et al. (1996) involving 96 boys under the age of 12 years and 64 boys and men aged 12 and over (with a full mutation).

Table 1.3: Proportion of affected boys with various medical problems

Problems	Proportion
Failure to thrive	15%
Recurrent vomiting	31%
Epileptic seizures	22%
Strabismus	36%
Hernia	15%
Recurrent otitis	85%

Source: Hagerman (1997a).

Actually, mental retardation is only one aspect of an overall developmental delay which starts early and affects both motor and cognitive development, language acquisition and social integration[12]. Language acquisition is often delayed, which is an important sign pointing to the diagnosis (Abbeduto and Hagerman, 1997). Subsequently, rapid, jerky speech with perseveration is fairly characteristic. The vocabulary and syntax are weak. The relational aspects of language are disturbed as well, with the affected individual often experiencing problems sustaining a conversation.

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[12] Bailey et al. (1998) observed, in 46 affected preschool-age boys, psychomotor development equal, on average, to that of children twice as young and a rate of development that was twice as slow.

1.3.2 Physical signs

The most recognizable clinical signs of fragile X syndrome are facial dysmorphia and macroorchidism. Characteristically, the face is elongated, with a high forehead and large, prominent ears, prognathism (prominent lower jaw), a high-arched palate and increased head circumference. Strabismus is frequent. The muscles may be hypotonic, which causes a lack of facial expression. In affected females, the facial morphology may resemble that of males, but more discreetly. Macroorchidism (abnormally large testicular size) usually appears during puberty and is present in most affected adult males. All of these clinical signs are less frequent before puberty. Table 1.2 shows the frequency of the main dysmorphic signs before and after puberty[13].

In young children, hypotonia may be detected upon clinical examination. The stages of development associated with fine and gross motor skills are delayed. The hypotonia is often accompanied by poor motor coordination and sometimes sucking and swallowing problems, which can lead to failure to thrive during the first few months. Most affected children also have recurrent otitis.

An unusual growth curve has also been observed, with above-average height during childhood, but not during adulthood, because of a weak growth spurt during puberty (Loesch, 1995). Other physical signs sometimes suggest the possibility of a connective tissue abnormality: flat feet, pectus excavatum, scoliosis, hernia, excessive joint laxity[14] and mitral valve prolapse. Lastly, the incidence of epileptic seizures is reportedly quite high in fragile X individuals. Table 1.3 summarizes the frequency of some of the problems that may require medical intervention.

1.3.3 Behavioural problems

Behavioural problems dominate during childhood and adolescence, especially in boys. They may dominate the clinical picture, relegating psychomotor delay to a position of secondary importance, and be the main reason for seeking consultation. Younger children exhibit irritability. Subsequently, the most common problems are attention disorders with or without hyperactivity. They reportedly occur, during childhood, in 50 to 80% of boys and 35% of girls but often regress after adolescence (Hagerman, 1996b; Hagerman, 1997b). However, impulsive and even aggressive behaviours may persist. The main behavioural features are summarized in Table 1.4 below.

Affected individuals generally exhibit behaviours indicative of poor social integration, such as eye contact avoidance, a tendency toward isolation, excessive shyness, anxiety and stereotyped behaviours, such as hand flapping and hand biting, and activity and speech perseveration. Although these autistic-like features are frequent, pronounced disinterest in social interactions is rarely observed[15].

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[13] Most clinical studies are prone to selection bias and to overestimating the frequency of the clinical signs used to select the children referred for a diagnostic investigation.

[14] Especially evident in the metacarpophalangeal joint in children.

[15] Several studies have explored the possible links between fragile X syndrome and autism, but the association between these two clinical entities is still debatable (Feinstein and Reiss, 1998; Hagerman and Lampe, 1999; Mazzocco, 2000). It would seem that the diagnostic criteria for autism are met in about 15% of fragile X males and that about 4 to 6% of autistic individuals have the syndrome (Hagerman and Lampe, 1999). This figure is comparable to the percentage of males with mental retardation of unknown etiology in whom the syndrome has been identified, which justifies the use of diagnostic tests in the autistic population.

Table 1.4: Proportion of fragile X males with various behavioural signs, by age.

Behavioural signs	< 12 years	≥ 12 years
Hyperactivity	89%	64%
Perseveration	95%	100%
Eye contact avoidance	88%	98%
Stereotyped behaviours involving the hands (e.g., hand flapping)	85%	81%
Anxiety	64%	79%
Tactile defensiveness	76%	86%
Violent, impulsive behaviours	25%	42%

Source: According to a study by Merenstein et al. (1996) involving 96 boys under the age of 12 years and 64 boys and men aged 12 and over (with a full mutation).

However, they are frequently characterized by ambivalence, with simultaneous approach and withdrawal movements. This may be associated with shyness and anxiety, but also with hypersensitivity to sensory stimuli, whether auditory, visual, olfactory or tactile (Cohen, 1995). In particular, this hypersensitivity leads to defensiveness and withdrawal in the presence of painless tactile stimuli. It also translates into intolerance toward environmental changes.

Given the strengths and weaknesses in their cognitive development, the features of their speech and the particular aspects of their behaviour, fragile X children are recognized as having a particular behavioural and neuropsychological profile (phenotype) (Baumgardner et al., 1994; Bennetto and Pennington, 1996; Flint, 1998; Livet et al., 1999). All of these features, not just cognitive impairment, have an impact on their adaptive skills. Of these, social and communication skills are especially affected (Dykens et al., 1993; Freund et al., 1995; Bailey et al., 1998). And these skills are precisely the ones that have an impact on the social integration of the mentally retarded (Bouchard et al., 1996). The problems that occur in this regard are therefore the disabilities that contribute, depending on the environment, to the development of a handicap in affected individuals (Bailey and Nelson, 1995).

1.4 DIAGNOSIS

A diagnosis of fragile X syndrome may be suspected in the presence of a suggestive phenotype and/or a family history of mental retardation whose transmission seems to be X-linked. However, no clinical sign is pathognomonic, and there are several other forms of nonspecific, X-linked mental retardation. The diagnosis can therefore not be confirmed on the basis of the clinical findings alone. Furthermore, a family history of mental retardation is not an indispensable criterion for making a diagnosis, since, in many cases, such history cannot be documented. The proportion of individuals in whom a diagnosis is made in the absence of a known family history of mental retardation varies from centre to centre according to the method of recruiting patients referred for diagnostic testing, but it can be as high as 35% (Cossée et al., 1997; Mornet and Simon-Bouy, 1996).

Up until 1991, cytogenetic analysis was the only means available for confirming a diagnosis (see Appendix II). Cytogenetic analysis consists in examining, under a microscope, chromosomes during metaphase (as is done for karyotyping). A fragile site is one where there is a constriction, a vacuole or sometimes a rupture of the chromosome. These abnormalities can be detected only in a certain percentage of cells (4 to 50%) and only under certain experimental conditions. The fragile X site is located on the long arm of the X chromosome (at Xq27.3). Its detection requires a cell culture in a medium with a low folic acid and thymidine content. This is followed by an examination of a large number of mitoses.

Precise instructions for detecting the fragile site have been published (Jacky et al., 1991) in order to standardize the procedure and establish positivity criteria for the test. It is a labour-intensive technique whose interpretation requires a certain level of expertise and which entails a number of problems. First, the percentage of cells in which the fragile site is detected can depend on various technical conditions, but also on the dietary ingestion of folic acid and possibly the individual's age. Second, other fragile sites have been documented near the FRAXA site. Not all of these sites can be distinguished from the FRAXA site during cytogenetic analysis. Some are therefore responsible for a certain number of false-positive results.

Since 1993, cytogenetic analysis has been gradually abandoned in favour of molecular analysis because of the limitations mentioned above, but especially because it can be used only to make a diagnosis of fragile X syndrome in affected individuals and does not help identify asymptomatic carriers (Oostra et al., 1993).

1.5 MEDICAL AND SOCIAL MANAGEMENT

No curative treatment is presently recognized as beneficial for individuals with fragile X syndrome. Because of the effect of folic acid on the *in vitro* expression of the fragile site, the therapeutic effect of folic acid has been investigated. Certain beneficial effects on behavioural problems were initially reported, but those effects were not confirmed by seven subsequent randomized trials based on standardized measurement instruments but involving a limited number of subjects (Murray et al., 1997).

Therefore, medical management of the syndrome[16] basically consists of a regular follow-up to prevent or monitor its usual clinical manifestations, such as hypotonia, otitis and behavioural problems. Pharmacologic treatments[17] for hyperactivity, attention disorders and anxiety may be indicated, depending on the extent of the behavioural problems, which are often the main concern of parents of affected school-age children (Hagerman, 1997b; Hagerman, 1999). Treating recurrent otitis is important for preventing hearing problems that would further delay language acquisition. Prophylactic antibiotic therapy is required for surgical interventions and dental care in the presence of mitral valve prolapse.

The American Academy of Pediatrics has issued recommendations, by age group, for following affected children both from a medical standpoint and from the standpoint of the guidance that the child and his or her family should receive (AAP, 1996). Although the regular follow-up and the coordination of medical care can be provided by pediatricians or general practitioners (Cooley, 1999), the child will need to be referred to various specialized clinics (neuropediatrics, pediatric psychiatry, ophthalmology, orthopedics, cardiology, etc.), depending on his or her age and the problems at hand. Furthermore, as soon as the diagnosis is made, the parents should be able to obtain genetic counselling (see Section 3.2).

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[16] For a more detailed description, see Hagerman (1996a, 1997b) and de Vries et al. (1998b).

[17] Different drugs may be used, depending on the age and predominant symptoms, but, thus far, very few controlled studies have compared the various pharmacologic approaches for lack of sufficiently large series of affected children whose behavioural problems have been well described (Hagerman, 1999).

Multidisciplinary palliative management[18] is desirable for promoting the school and social integration of affected children, and early intervention is required if one wishes to optimize their developmental potential[19]. The children's needs vary considerably according to their age, the predominant symptoms and the degree of cognitive impairment. The involvement of various professionals is required: speech therapists, occupational therapists, psychologists, physiotherapists and special education teachers. A developmentally delayed child under the age of 5 years reportedly requires about four to five hours of direct, professional guidance (all professions combined) and four to five hours of indirect, professional guidance per week[20]. Since the bulk of the child's day-to-day management is nonetheless provided by the parents, the latter also require information, support (psychological[21], respite, etc.) and assistance in seeking out the appropriate services.

During the preschool years, occupational therapy, physiotherapy, speech therapy and psychological services are often required to meet the child's needs. A psychologist's intervention involves an accurate assessment of the child's cognitive development and behavioural adaptation. The assessment is often complicated by the attention problems and hyperactivity that some children exhibit as early as the age of 3 years. Also, the difficulty they have in dealing with new situations can cause such shyness and anxiety that there is a risk of underestimating their performance. Nonetheless, each child's abilities should be accurately evaluated on a regular basis in order to develop an individualized service plan. Speech therapy is generally required early because of the delay in language acquisition, and, in more severe cases, physiotherapy may be indicated for motor and feeding problems as early as the first year of life. Occupational therapy helps motor development and helps stimulate the child. All of the professionals involved also help encourage and train the parents to continue this stimulation at home by means of appropriate exercises and games.

The type of intervention recommended in terms of speech therapy and occupational therapy has not been evaluated specifically for fragile X syndrome, but such therapies have been used in children with similar problems (for example, autism or delayed speech due to mental retardation) (Scharfenaker et al., 1996; Abbeduto and Hagerman, 1997). As for the recommended approaches, much importance is attached to sensory integrative approaches[22] and to the offer of integrated services in the form of joint care by a number of functional reeducation professionals or in the form of ongoing collaboration[23] (Wilson et al., 1994; Scharfenaker et al., 1996).

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[18] For further details, see Wilson et al. (1994) and Scharfenaker et al. (1996).

[19] No study specific to fragile X syndrome has documented the effect of "early intervention" on these children's long-term prognosis (Bailey et al., 2000). The recommendations in this regard are based on the accumulated experience of a few specialized centres and on the literature on early intervention in developmentally delayed children. Although, on the whole, this literature has demonstrated the efficacy of early multidisciplinary interventions with the parents' involvement, some authors believe that one cannot justifiably extrapolate the conclusions in this literature, which is largely based on studies involving children whose developmental delay could have had an environmental cause, to children whose developmental delay may be of biological origin (see discussion in Parry, 1992). However, the efficacy of early intervention has been documented for two groups of children with problems similar to those observed in fragile X children: children with delayed language acquisition and autistic children. Also, the objective of these programs is not only to optimize the children's development, but also to prevent secondary handicaps (e.g., behavioural problems exacerbated by speech and communication problems) and to give the parents support that will enable them to deal with their children's special needs (Parry, 1992).

[20] As determined by the Centre de Réadaptation de l'Estrie (CRE).

[21] The syndrome's hereditary mode of transmission gives rise to a particular psychological setting that should be taken into consideration when offering individualized family support (Bailey and Nelson, 1995). Moreover, psychological support for parents of children with behavioural problems in addition to mental retardation is especially important, given that these difficulties constitute an additional source of stress for the parents and because maladaptive behaviours are a major obstacle to school and social integration (including, later on, workplace integration) (Bouchard et al., 1996).

During the school years as well, each child's abilities should be evaluated on a regular basis so that he or she might receive the supervision best suited to his or her cognitive development and behavioural profile. Although most affected boys will clearly not be able to meet the requirements of a normal curriculum, there is no consensus as to the type of educational path that should be chosen, and this path should, no doubt, depend on each child's strengths and weaknesses. Whatever the school setting, affected children will exhibit, to varying degrees, learning difficulties that will require special support from speech therapists or special education teachers, depending on the case. No rigorous studies involving a sufficient number of children with this syndrome have been conducted to demonstrate which educational strategies could promote the optimal development of their abilities (Bailey and Nelson, 1995). However, based on the expertise accumulated at specialized centres, it would be highly desirable, in addition to providing specialized services (speech therapy, occupational therapy, etc.), to take into account the developmental and behavioural features specific to the syndrome when adapting the learning strategies and to modify the children's environment accordingly (Scharfenaker et al., 1996). Table 1.5 summarizes a few recommendations in this regard.

When children reach adolescence, new concerns arise: their autonomy with regard to daily activities, their preparation for professional life and their participation in social life. In general, parents continue to look after their children when they are adults, but housing may become a problem if the parents are no longer able to provide it. In such cases, various forms of assisted living may be considered[24]. Depending on their level of autonomy and their behavioural characteristics, adults may be channeled into different work integration programs[25].

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[22] The use of these approaches is based on the assumption that problems integrating sensory stimuli, but also proprioceptive and vestibular stimuli, may be at the origin of attention disorders, which affect cognitive development, but also of certain symptoms (tactile defensiveness, eye contact avoidance, stereotyped behaviours, etc.) that affect communication and social skills.

[23] The advantage of providing integrated services is illustrated by the following two examples: 1) language acquisition requires adequate tonus of the trunk and neck and sufficiently developed facial muscle motricity and coordination; and 2) the use of certain rhythmic movements or tactile stimuli (deep rather than superficial) could improve speech delivery and concentration and therefore prepare the children for various other learnings.

[24] Residential resources include group homes, supervised apartments and foster homes. The little support provided to the family home is regrettable.

[25] True work integration is seldom achieved by individuals with mental retardation. Behavioural problems make such integration even more difficult (Ministère de l'Éducation du Québec, 1999b). Some individuals enter subsidized work integration contracts (OPHQ), but they rarely lead to integration. Others do workplace traineeships, which may or may not be supervised and which are not always remunerated. Lastly, a certain number attend sheltered workshops.

Table 1.5: Examples of adjustments made to learning strategies and of environmental changes that can have a beneficial effect according to the cognitive and behavioural characteristics of fragile X children

Cognitive/ behavioural characteristics	Recommended strategies
Hypersensitivity to sensory stimuli	Avoid visual and auditory distractions. Small groups. Do not force eye contact. Postural stabilization.
Difficulties concentrating	Adjust length of activities. Provide calming or sensory integration activities before the learning activities.
Speech problems	Allot more time. Avoid any source of anxiety.
Difficulties with novelty and with transition between activities	Staff continuity. Regular, predictable schedules. Prepare transition between activities.
Difficulties with sequential activities	Simple and concise instructions. General presentation of tasks and objectives.
Difficulties with abstraction and generalization	Adapt the curriculum to include simple and concrete activities that relate to daily tasks.
Fine motor problems	Use technical aids (calculators, computers, etc.).
Imitation and visual memory skills	Activities with peers who can serve as speech and behaviour models. Combine visual and auditory cues.

Source: Inspired by Wilson et al. (1994) and Scharfenaker et al. (1996).

2. GENETIC ASPECTS OF FRAGILE X SYNDROME

It is now well established that fragile X syndrome is associated with mutations of the FMR1 gene and that this gene contains the region identified as a fragile site upon cytogenetic analysis[26]. Different convergent avenues of research have contributed to these discoveries and to an understanding of the mechanisms involved in the mutations. These complex steps are briefly discussed in Appendix I.

The development of molecular tests, which can be used to identify mutations in affected individuals and asymptomatic carriers, and our knowledge regarding the correlation between the clinical expression (the phenotype) and the nature of the mutations (the genotype) constitute a major contribution to genetic counselling. Nonetheless, the limits of our current knowledge have an impact on genetic counselling, which makes decisions in the context of prenatal diagnosis especially complicated.

2.1 THE FMR1 GENE

The FMR1 (fragile X mental retardation 1) gene was identified in 1991 (Verkerk, 1991). It contains 17 exons (coding sequences), and the FRAXA site is located in the untranslated part of the first exon.

The fragile site is actually a short DNA sequence containing a repeat of a variable number of CGG (cytosine-guanine-guanine) triplets interspersed throughout with a few AGG (adenine-guanine-guanine) triplets. This polymorphic site usually contains between 6 and 54 triplets (average of 29 or 30 triplets), but there can be as many as a few thousand repeats in fragile X individuals. The alleles are characterized by the number of repeats, but also by the presence or absence of methylation of this region of the gene, for methylation of the trinucleotide repeat itself and of a sequence rich in cytosine-phosphate-guanine dinucleotides located near the repeat is believed to play a role in the gene's expression. This sequence, called the CpG island[27], is normally methylated on the inactive X chromosome in females.

In addition to this mutation, which is characterized by an expansion of the number of triplets and now referred to as a "dynamic mutation", a few deletions and point mutations in the FMR1 gene have been identified[28], but they probably account for fewer than 2% of the mutations of this gene. A few of them are new mutations, although thus far, all mothers of children with a dynamic mutation have also carried an expansion of the trinucleotide repeat (Snow et al., 1993; Reiss et al., 1994).

2.2 DYNAMIC MUTATIONS

Dynamic mutations, first documented in fragile X syndrome, are now recognized as a class of mutations involved in several hereditary diseases[29], such as myotonic dystrophy and Huntington's disease. The dynamic nature of these mutations translates into variations in allele size during cell divisions: meiotic instability, which explains the particular hereditary mode of transmission of these diseases, and mitotic instability, which explains the high proportion of somatic mosaics observed in affected individuals.

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[26] A somewhat more detailed discussion of the genetic aspects of the syndrome can be found in the articles by Nussbaum and Ledbetter (1995), Brown (1996), de Vries et al. (1998b), Eichler and Nelson (1998) and Kaufman et al. (1999).

[27] This sequence is part of the gene's promoter region, which plays a role in the regulation of the gene's expression and therefore in the production of the protein the gene codes for.

[28] The other types of mutations described in the FMR1 gene include point mutations (De Boulle et al., 1993; Lugenbeel et al., 1995), a splice mutation (Wang et al., 1997), total and partial deletions and mosaics combining deletions and dynamic mutations (Gedeon et al., 1992; Wöhrle et al., 1992; Tarleton et al., 1993; Gu, et al. 1994; Meijer, et al. 1994; Trottier et al., 1994; van den Ouweland et al., 1994; de Graaff et al., 1995; Hirst et al., 1995; Quan et al., 1995; Mannermaa et al., 1996; Milà et al., 1996; Hammond et al., 1997; Parvari et al., 1999; Petek et al., 1999; and García Arocena et al., 2000).

In fragile X syndrome, the alleles are classified into three categories, based on the number of repeats that characterize them and on their methylation status (Table 2.1). Thus, there are normal alleles (containing between 6 and 54 triplets), premutated alleles (containing between 55 and 199 triplets) and full mutations (of more than 200 trinucleotide repeats). In reality, there is overlapping between the size of premutated and mutated alleles. The distinction is therefore made mainly on the basis of the methylation status. Full mutations are methylated, and it is they that give rise to clinical signs of the disease, whereas premutation carriers are basically at risk for transmitting the syndrome to their offspring.

Normal alleles are transmitted in a stable manner from generation to generation. Only small size differences may be observed. However, there is a grey zone, whose alleles contain 45 to 54 repeats and whose behaviour is less well known[30]. Premutations are unstable (the number of repeats can increase or, rarely, decrease) during female meiosis, with the result that a premutation can expand into a full mutation when transmitted from a woman to her children. In affected families, about 70 to 80% of the premutations will expand into full mutations during mother-child transmission (Fu et al., 1991; Heitz et al., 1992; Fisch et al., 1995; Sherman et al., 1996; Murray et al., 1997), but the probability of expansion depends on the size of the premutated allele (Fu et al., 1991; Yu et al., 1992; Heitz et al., 1992; Snow et al., 1993). During male meioses, premutations are generally transmitted in a more stable fashion[31] (Snow et al., 1993). Full mutations are unstable and exhibit a tendency to expand during each generation-to-generation transmission ("meiotic" instability).

Full mutations are also unstable during cell divisions in a given individual, and this "mitotic" instability causes differences in allele size between cell lines. This heterogeneity is all the more pronounced because the expansions are large, but it is sometimes observed in the case of large premutations.

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[29] The implication of a dynamic mutation has now been documented in at least a dozen hereditary diseases, especially in the field of neurogenetics.

[30] Grey zone alleles may be slightly unstable, but no expansion, in a single generation, from an allele of fewer than 55 repeats into a full mutation has been documented (Hirst, 1995; Zhong et al., 1996; Murray et al., 1997).

[31] A decrease in the size of the expansion was observed in about 30% of the male meioses (Fisch et al., 1995), and unlike female meioses, the probability of premutations expanding during male meioses is inversely associated with the size of the premutation (Ashley-Koch et al., 1998). This observation and the observation of premutations in the sperm of males with full mutations has led to the hypothesis of selection against sperm carrying a full mutation.

Table 2.1: Summary of the main alleles of the FMR1 gene

	Number of triplets	Methylation status
Normal alleles	6 –54	-
Grey zone alleles	45 – 54	-
Premutations (PMs)	55 – 200	-
Full mutations (FMs)	> 200	+
Allelic mosaics	PMs + FMs	+/-
Methylation mosaics	> 200	+/-

The existence of two types of mosaics makes interpreting molecular tests more complicated. Allelic mosaics result from mitotic instability and include both premutations and full mutations[32]. One multicentre study documented this type of mosaic in about 12 to 15% of the full mutations in males and 6 to 7% in females (Rousseau et al., 1994). Other studies have reported substantially higher rates (up to 40%). These differences are perhaps attributable to technical differences in the molecular tests (Snow et al., 1993; Kaplan et al., 1994; Nolin et al., 1994; Youings et al., 2000). A methylation mosaic is where the degree of methylation varies from one cell line to another in certain individuals with a full mutation. The proportion of cells whose mutation is methylated varies considerably (0 to 95%, according to Steyaert et al., 1996). This type of mosaic is more rare.

2.3 THE GENE PRODUCT

The FMR1 gene is translated into a protein called "FMRP" (fragile X mental retardation protein). In most tissues, this protein is present in minute quantities, although it is more easily detectable in the central nervous system and testicles. The fact that the syndrome can result from a point mutation in the FMR1 gene confirms that the clinical manifestations result from a lack of functional FMRP. Although the role of this protein has not yet been completely elucidated, recent research has shown its association with ribosomes in the cytoplasm (Khandjian et al., 1996), which suggests that it could play a role in protein synthesis.

When there is no CpG island methylation, i.e., in the presence of normal alleles or premutations, expression of the gene translates into FMRP synthesis. However, a full mutation is most often accompanied by total CpG island methylation and the absence of mRNA synthesis (Pieretti et al., 1991; Sutcliffe et al., 1992) and therefore of FMRP synthesis (Devys et al., 1993; Verheij et al., 1993). In methylation mosaics, variable quantities of FMRP can be detected, as in allelic mosaics.

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[32] There are also rare cases of allelic mosaics including normal alleles, in addition to full mutations (Snow et al., 1993; van den Ouweland et al., 1994; Rousseau et al., 1994; Nolin et al.,1994; Haddad et al., 1996; Milà et al., 1996; Mingroni-Netto et al., 1996; Perroni et al., 1996; Orrico et al., 1998; Schmucker and Seidel, 1999; and Gold et al., 2000) or deletions (see Section 2.1).

2.4 PHENOTYPE/GENOTYPE CORRELATION

The main aspects of phenotype/genotype correlation are well documented for fragile X syndrome, but there are still major gaps in our knowledge, and they have a considerable impact on phenotype prediction at the individual level.

Classical clinical expression, as described in Section 1.3, is due to the presence of a full mutation. Almost all males who carry a full mutation present with some of the components of this clinical picture, whereas females who have inherited a full mutation exhibit mental retardation in only about 55% of cases.

However, a small proportion of males with a full mutation apparently have an IQ within normal limits, i.e., above 70. These males, referred to as "high-functioning males", may nonetheless exhibit dysmorphic signs or behavioural features of the syndrome (McConkie-Rosell et al., 1993; Hagerman, 1994a; Steyaert et al., 1996; Merenstein et al., 1996). They generally have allelic or methylation mosaics of which most cells carry an unmethylated full mutation (Hagerman, 1994a). On average, males with mosaics—allelic and methylation—reportedly have an IQ above the average observed for those with a completely methylated full mutation (Table 2.2). In prepubertal boys, the differences in terms of the type of mutation seem less pronounced, with the mean IQ for a completely methylated full mutation being higher (51.3; min.-max.: 30-80). The proportion of high-functioning males among full mutation carriers has been estimated, by Hagerman et al. (1994a), at 13%. However, their study involved mainly prepubertal boys, and a substantial decrease in IQ was observed in most of those who were evaluated a second time. Upon the subsequent follow-up of this group, it seems that the proportion of boys who maintained, until adolescence or adulthood, an IQ above 70 fell to 2.8% (Hagerman, 1995)[33].

In females with a full mutation, phenotype prediction poses a much greater problem because of the variability in the clinical manifestations (Nussbaum and Ledbetter, 1995). Although it is generally considered that about 55% of females with a full mutation are mentally retarded, this figure does not, perhaps, reflect the actual extent of the problems encountered in affected females[34] According to de Vries et al. (1996), approximately 50% of them have an IQ below 70, but about another 20% could have a borderline IQ of between 70 and 85 The mean IQ varies considerably from study to study but could be approximately 75 to 85 (Bennetto and Pennington, 1996; de Vries et al., 1996). Also, females with a full mutation and a normal IQ may nonetheless have learning difficulties (attention deficit), behavioural problems (anxiety, impulsiveness, avoidance behaviours) and reduced performance for certain cognitive functions (executive functions) (Lachiewicz, 1995; Mazzocco, 2000).

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[33] Since underdiagnosis might be more pronounced in high-functioning males than in other carriers of full mutations, it is difficult to estimate the exact prevalence.

[34] For an overview of the frequency of the physical signs and behavioural characteristics, see Riddle et al. (1998).

Table 2.2: IQ of affected males (> 12 years) according to the type of mutation

Type of mutation	**N**	**Mean IQ**	**Min-Max**
Completely methylated full mutation	51	41.2	15-66
Allelic mosaic	17	60.1	40-92
Methylation mosaic with < 50% methylation	4	88.2	73-100

Source: Merenstein et al. (1996).

This variability in expression and the lower degree of severity of the syndrome are attributed to the fact that females also have an X chromosome that does not carry the mutation. Indeed, the clinical expression seems to be linked to the preponderant inactivation of the X chromosome carrying the mutated allele in the target organs (Reiss et al., 1995). The results of all the studies of correlations between the activation ratio in leukocytes[35] and the clinical signs are not in complete agreement (Murray et al., 1997; Kaufmann et al., 1999). Research is also underway on FMRP expression in various tissues and its link with clinical expression (Tassone et al., 1999; Willemsen et al., 1997b, 1999). Presently, neither of the two methods is ready to be used in a clinical setting to determine the phenotype of females with a full mutation (Kaufmann et al., 1999). The difficulty in predicting pathological effects in females carrying a full mutation is therefore an important problem for genetic counselling and especially for decision-making during prenatal diagnosis.

Premutation carriers are usually asymptomatic, whether females or normal transmitting males. An association between positive premutation carrier status and a tendency toward premature ovarian failure has, however, been described in certain families (Allingham-Hawkins et al., 1999), but the underlying pathogenesis remains a mystery. More recently, the possibility of less suggestive manifestations (anxiety, learning disabilities, attention deficit, impulsive tendencies) in a small proportion of premutation carriers has been raised (Loesch et al., 1994; Hagerman et al., 1996; Murray et al., 1996; Riddle et al., 1998; Youings et al., 2000), but this is still debated[36] (Mazzocco et al., 1997; Crawford et al., 1999; Mazzocco, 2000).

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[35] Inactivation of one of the two X chromosomes occurs at random during the development of female embryos. Since variations in X chromosome inactivation ratios have been observed in different tissues (Azofeifa et al., 1996), the activation ratio in leukocytes, i.e., the proportion of these cells in which the active chromosome carries the normal gene, does not necessary reflect the situation in the central nervous system.

[36] The debate is fueled by recent publications concerning mRNA synthesis and FMRP synthesis in premutation carriers. In 1995, Hagerman (1995) hypothesized that there is a continuum of physical, cognitive and behavioural manifestations associated with FMRP expression that results from the different molecular characteristics, such as expansion size, the degree of methylation and the chromosome X inactivation ratio.

2.5 MEIOTIC STABILITY OF THE ALLELES

The meiotic stability of the alleles was investigated as soon as mutations in the FMR1 gene were discovered, given that the assessment of the risk of premutations expanding into full mutations determines the need for prenatal diagnosis. For women carrying a premutation, the risk of transmitting the syndrome consists of the risk of transmitting the mutated gene (50%) plus the risk of the premutation expanding into a full mutation. Small premutations are more stable than larger ones, whose number of repeats generally increases during transmission to the next generation (Fu et al., 1991; Heitz et al., 1992; Snow et al., 1993; Fisch et al., 1995).

Nonetheless, assessing the expansion risk for a given premutation is difficult because the risk of a premutation becoming a full mutation depends not only on its size, but also on the number of AGG triplets interspersed in the segments of CGG triplets (Eichler et al., 1994). It is reported that AGG triplets stabilize the sequence and that the critical threshold for expansion is an uninterrupted series of more than 33 CGG triplets (Eichler et al., 1994; Zhong et al., 1996; Eichler and Nelson, 2000).

Murray et al. (1997) performed a meta-analysis of five retrospective studies and developed a regression model for estimating the probability of a maternal premutation expanding into a full mutation in the child from the size of the premutation. These data[37], which are summarized in Table 2.3, show that the risk increases very rapidly and support the usual practice of proposing prenatal diagnosis whenever the genotypic analysis of the mother reveals a premutation. One prospective, multicentre study of the expansion risk has not yet followed enough cases by stratum to determine this risk more accurately and to reduce the size of the confidence intervals surrounding these estimates[38].

As for grey zone alleles[39], rare cases of intergenerational instability[40] have been documented for alleles with 46 to 54 repeats (Reiss et al., 1994), but no allele of fewer than 55 repeats has become a full mutation in a single generation (Hirst, 1995; Zhong et al., 1996; Murray et al., 1997).

Lastly, the available expansion risk data are based on mother-child transmissions observed in families with a family history of the syndrome. Yet, one Québec study showed that the prevalence of premutations in pregnant women is 1 in 259 (95% confidence interval: 1/373 to 1/198)[41], if no selection is done on the basis of family history (Rousseau et al., 1995).

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[37] The data presented in Table 2.3 have not been corrected for the selection bias potentially associated with the recruitment of families through index cases.

[38] In 83 cases of transmission of a premutated maternal allele, expansion into a full mutation was observed in the child. The following expansion risks (and 95% confidence intervals) can be calculated from the data: 0% (0-50) for alleles of 50 to 59 triplets; 37% (4-70) for alleles of 60 to 69 triplets; 91% (74-100) for alleles of 70 to 79 triplets; 63% (39-87) for alleles of 80 to 89 triplets; and 98% (93-100) for maternal alleles of more than 90 triplets (Sherman et al., 1996).

[39] These alleles, which contain between 45 and 54 triplets, reportedly account for about 1 to 2% of the alleles found in the population (Snow et al., 1993; Reiss et al., 1994; Dawson et al., 1995; Holden, 1995a; Brown et al., 1996).

[40] About 50% of grey zone alleles are reported to contain uninterrupted stretches of more than 33 triplets (Zhong et al., 1996).

[41] This high prevalence could be the result of a founder effect (Sherman, 1995). Two previous studies, which involved smaller samples, had found a premutation in 1 in 197 females (Snow et al., 1993) and in 1 in 561 females (Reiss et al., 1994), respectively.

Table 2.3: Risk of expansion of a maternal premutation into a full mutation in the child according to the size of the premutation

Size of maternal PM	Risk of expansion into a FM (%)	95% confidence interval (%)
60	23	6 – 57
65	34	11 – 70
75	62	27 – 88
80	74	40 – 93
85	84	54 – 96
95	95	79 – 99
100	97	87 – 100
105	99	92 – 100

Source: Murray et al. (1997).

Consequently, studies are being carried out to determine the expansion risk for females with no family history of the syndrome, since such data are necessary for estimating the risk of expansion when a premutation has been identified by chance (for example, the spouse of a normal transmitting male) and for determining the usefulness of conducting more-systematic screening of low-risk females (see Section 6.2).

2.6 RECAP

Considerable advances have been made in a few years with regard to our knowledge of the gene, the dynamic mutation and FMRP. This knowledge has no doubt resulted in fragile X syndrome being the most widely known cause of mental retardation (Kaufmann et al., 1999), and has significantly changed diagnosis and genetic counselling, as discussed in the next few chapters. In addition, the distinction between the different types of mutations has permitted a finer description of the cognitive and behavioural phenotype, which should lead to more-rigorous studies of pharmacological, educational and psychosocial interventions over the next few years (Mazzocco, 2000). However, a number of aspects of the phenotype/genotype correlation and of allelic stability have not yet been completely elucidated and are, to a certain extent, obstacles to the optimal application of the knowledge derived from molecular genetics to clinical practice.

3. THE CONTRIBUTIONS OF MOLECULAR GENETICS

Since 1991, when the gene was localized and the mutation identified, genotypic analysis has been used to make a diagnosis in symptomatic individuals, determine the mutation carrier status of an affected individual's family members and, if applicable, to perform a prenatal diagnosis. Molecular analysis has replaced cytogenetic analysis, which did not permit identification of premutations and therefore of asymptomatic carriers of a mutated gene. The possibility of accurately characterizing the type of mutation has made genetic counselling considerably easier, as explained in Section 3.2. The indications for molecular testing for diagnostic purposes are still the subject of some debate, which we discuss in Section 3.3[42] before presenting, in Section 3.4, the molecular diagnostic protocols from the standpoint of the techniques used. However, we will first examine the hereditary mode of transmission of fragile X syndrome, which molecular genetics has elucidated.

3.1 THE CONTRIBUTION OF MOLECULAR GENETICS TO UNDERSTANDING THE HEREDITARY MODE OF TRANSMISSION

Molecular genetics has, to a large extent, elucidated the syndrome's enigmatic mode of transmission. The syndrome follows an X-linked dominant mode of transmission, which implies that it can manifest from the time a single copy of a full mutation is present and that both females and males can therefore be symptomatic. However, the syndrome's penetrance is incomplete, given that up to 20% of males and about 65% of females who are obligate carriers do not exhibit any mental retardation (Sherman et al., 1985). It exhibits variable expressivity both in males and in females, who are, on the whole, less severely affected.

The distinction between premutations and full mutations is based mainly on the number of trinucleotide repeats, but above all on the methylation status of the FMR1 gene. The existence of males and females who are obligate carriers but nonetheless asymptomatic can be explained by the fact that they carry unmethylated premutations. The most thorough study of the pathologic mechanisms suggests that the mutated gene's penetrance is a function of its degree of methylation in the target tissues and, in females, of the inactivation of the X chromosome carrying the mutation. As for the syndrome's expressivity, it might also depend on the degree of methylation of the mutated gene, which conditions FMRP synthesis, but a firm correlation has thus far not been established. Our understanding of the dynamic nature of the mutation and the distinction between premutations and full mutations, and research on the instability of premutated and mutated alleles, have also helped explain the phenomenon of anticipation and Sherman's paradox.

Within a given family, there are individuals with normal alleles, premutations and full mutations. A women with a premutation has a 50% chance of transmitting the mutated gene to each of her children, but the premutation can also become a full mutation during the process. The probability of this occurring is at least 10 to 20% for the smallest premutations and increases with the size of the premutation (see Section 2.5). An asymptomatic woman can therefore give birth to affected children. Normal transmitting males transmit their premutation to all of their daughters[43], who, in turn, can give birth to children with a premutation or a full mutation.

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[42] However, strategies for identifying affected individuals and potential carriers are discussed in Sections 6.1 and 6.2, respectively.

[43] However, recently, a few cases of transmission of full mutations by normal transmitting males to their daughters have been described (Brown et al., 1995; Ventura et al., 1999; Lazarou et al., 1999; Bridge et al., 1999), raising

Females who have inherited a full mutation are symptomatic in only about 55% of cases and have a 50% chance of transmitting the mutated gene to their offspring. This implies that their sons will have a 50% chance of being affected, while for their daughters, the figure is around 28% (a 50% chance of inheriting the mutated gene multiplied by a 55% chance of exhibiting mental retardation if they inherited it) (Fu et al., 1991).

The phenomenon of anticipation[44] can be explained by the fact that the size of premutations tends to increase over the generations, which results in an increased risk of their becoming full mutations in successive generations. Since a full mutation very rarely regresses to a premutation, the probability of there being several affected individuals in a given generation increases from one generation to the next if no preventive measures are taken. This phenomenon also explains why the syndrome can appear in families with no family history of mental retardation, even if no *de novo* mutations have been documented to date.

Sherman's paradox derives from the fact that females who are asymptomatic but obligate carriers of the mutation actually constitute a heterogenous group consisting of females with a premutation and others with a full mutation. The chances of these two groups of females having symptomatic children are very different. The mothers of normal transmitting males are obligate carriers of a premutation, whereas the mothers of affected children may be carriers of full mutations or premutations. The former will therefore have, on average, fewer affected children than the latter.

In short, the characterization of the types of mutations in the FMR1 gene has contributed to understanding the particular aspects of the syndrome's hereditary mode of transmission that did not fit well with an X-linked dominant mode of transmission, such as the existence of males and females who are obligate carriers but nonetheless asymptomatic, the phenomenon of anticipation and Sherman's paradox. Research is continuing in an attempt to better explain the underlying mechanisms of the syndrome's expressivity and penetrance and the instability of mutated alleles.

3.2 THE CONTRIBUTION OF MOLECULAR ANALYSIS TO GENETIC COUNSELLING

Apart from our understanding of the hereditary mode of transmission, the major contribution of the molecular genetics of fragile X syndrome has been the development of direct molecular tests that can be used not only to confirm a diagnosis in affected individuals, but also to identify asymptomatic carriers in affected families or in at-risk populations. These tests have significantly altered the scope of genetic counselling.

3.2.1 The approach of medical genetics and practice guidelines

Given the complexity of the transmission and expression of fragile X syndrome and the difficult situations and choices facing the individuals concerned, diagnosing and screening should be carried out by professionals who are competent and sufficiently qualified to provide all the necessary information and answer the questions and meet the needs of individuals to whom testing is offered. Providing adequate assistance to these individuals or families requires an approach that incorporates both medical and technical skills and the skills required to deal with the psychological and ethical problems posed by genetic diagnosis.

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new questions about the indications for prenatal diagnosis (see Section 6.1.2).

[44] The phenomenon of anticipation here means that increased penetrance of the syndrome (a higher percentage of symptomatic individuals), not increased expressivity (greater symptom severity), as is the case in other diseases caused by dynamic mutations, such as myotonic dystrophy, occurs over successive generations.

The approach developed by medical genetics, which is based on genetic counselling, is probably the most adequate framework for achieving this complex task. The practice standards, developed by genetics professionals, take into account the fundamental principles of medical ethics and principles concerning the particular aspects of the familial nature of genetic information and the reproductive decisions based on it (Wertz and Fletcher, 1989; Caulfield et al., 1995). These standards have been incorporated into numerous national and international declarations and guidelines aimed at regulating, more generally, the use of genetic tests. These guidelines are all the more important because of newly emerging situations in which molecular tests can be offered. Fragile X syndrome is an example of the diffusion of this technology in various medical specialties, given that the diagnostic workup may be initiated for several different symptoms and therefore by various professionals. While genetic counselling is a practice traditionally associated with medical genetics, the observance of practice standards is not a given in all of these specialties.

The offer of molecular testing should be accompanied by genetic counselling in order to:

a) Assess the risk of transmission of the disease within a family on the basis of both a pedigree analysis and the results of clinical examinations and laboratory tests.

b) Provide, in an appropriate manner, information on the disease and the intervention options, with an explanation of the nature and prognosis of the disease, the available diagnostic and therapeutic modalities and their limitations, the risk of transmission to the following generations and the means of preventing a recurrence of the disease, taking into account the individual's or couple's ability to understand.

c) Support the individual or couple in his, her or their decision-making process and choices.

d) Maintain prolonged contact with the families in order to meet the request of each new member willing to receive genetic information and of couples who wish to have children.

All this should be done in accordance with basic ethical principles, i.e., respect for the dignity and autonomy of the person, the right to complete information and absolute confidentiality, beneficence, non-maleficence and justice, principles that are also inherent in medical ethics (WHO, 1998). Practice standards specific to genetic counselling are aimed at ensuring that participation in such counselling is voluntary and that it is conducted in a nondirective manner and at promoting the communication of information at a level that is appropriate for the patient, providing the necessary support for decision-making and supporting the individuals in their choices. The obligations of geneticists are not limited to diagnosis and treatment, but also include not abandoning the patient and his or her family and providing monitoring, follow-up and referrals to the appropriate services (Sharpe, 1996).

The mental retardation associated with fragile X syndrome raises additional ethical issues, such as the stigma associated with mental retardation, and leads to further difficulties for genetic counselling, such as making decisions in the presence of reduced cognitive abilities (Nuffield, 1998). Understanding the information, which is quite complex as it is, about the mode of transmission and the impact of the syndrome can be complicated by the psychological reactions of denial, guilt, anger, loss of self-esteem and depression that often occur upon communicating a diagnosis of mental retardation in the family (Partington, 1986). This situation requires openness to the problems and specific needs of individuals in affected families (Staley-Gane, 1996). Genetic counselling must sometimes be provided to female carriers with reduced cognitive abilities. This requires additional decision support, which can sometimes involve other family members (Watkins et al., 1989; de Vries et al., 1999b).

The emotional load of being informed that one's child is mentally retarded and the strong possibility of the stigma associated with this have a significant impact on the family. They can have a psychological impact or an impact on the family's dynamics and quality of life, and on the provision of relevant information to the members of the extended family who are at risk (Cronister, 1996; McConkie-Rosell et al., 1995; van Rijn et al., 1997). When providing information to family members poses a problem, the genetic physician or counsellor is faced with a conflict between the obligation to respect confidentiality and that of beneficence toward the relatives. Situations where it would be acceptable to breach confidentiality are still hotly debated. Also, because of the rapid pace at which our knowledge of fragile X syndrome is evolving, as is the case for many other hereditary conditions, providing families with updates on the information they receive would be desirable, although logistically, this aspect of clinical practice would be relatively unwieldy.

Despite the difficulties inherent in this delicate and complex task, the molecular genetics of fragile X syndrome has made genetic counselling of individuals concerned by it much easier. This contribution is assessed differently according to whether or not there is a family history of the syndrome. Two situations can thus be identified: genetic counselling of the members of an affected family and genetic counselling of couples with a low *a priori* risk. In the former case, the counselling is intended for the parents of an affected child or the at-risk relatives.

3.2.2 Genetic counselling of members of an affected family

In general, in clinical practice, the presence of fragile X syndrome in a given family is confirmed by molecular diagnosis of the syndrome in a symptomatic individual or by determining the carrier status of a woman of child-bearing potential with a family history of mental retardation or other signs associated with the syndrome.

Confirming the diagnosis in a symptomatic individual has direct benefits for this person, indirect benefits for the nuclear family and direct benefits for the nuclear family and extended family. Such benefits are associated with the obtaining of genetic counselling and the determination, within the framework of cascade screening, of their genotypic status. With molecular testing, this status can be determined with certainty, unlike with cytogenetic analysis, in which there was an appreciable margin of error. An accurate determination of the type of mutation not only confirms or rules out the existence of a risk for the offspring, but it also permits a more accurate assessment of the risk of transmission.

The impact anticipated when making an accurate diagnosis in affected individuals is adequate medical, psychosocial and educational management. The parents' anxiety is reduced when the source of the problem is identified and when they are referred to the appropriate resources (Carmichael et al., 1999; Roy et al., 1995). In addition, with a more complete evaluation of the nuclear family, one can verify the genotypic status of the other children and assess the risk of recurrence of the syndrome, and hence promote more informed reproductive choices. Since the mother has already given birth to an affected child, she is an obligate carrier of a premutation or a full mutation, and, in either case, the risk of maternal transmission of the syndrome is high. Various family planning options can then be discussed, taking into account the parents' experience, values and perception of the risk. Prenatal diagnosis might be proposed, if this seems useful. In this regard, the contribution of molecular testing is major, for it substantially reduced the uncertainty that prevailed with cytogenetic testing. In determining the type of mutation in the mother and fetus, options previously considered, such as deciding not to have any other children or deciding to have girls only, not knowing whether or not they would be affected (Partington, 1986), can now be rejected in favour of more-informed decisions[45] consistent with the couples' values.

Another purpose of genetic counselling is to inform the parents of an affected child (index case) of the risk to the extended family, given the syndrome's particular mode of transmission and expression. The brothers and sisters of the index case's mother who so request can then obtain genetic counselling and be screened. Carrier screening is performed in order to identify the individuals at risk for transmitting the syndrome to their offspring. Lastly, broader family screening is also possible, by determining the status of the index case's maternal grandparents, which enables one to target the collateral branch of the family to be screened. At-risk couples of childbearing potential can thus have unaffected children, if they wish. However, this is possible only if the index case's parents give the information to the relatives in a timely manner. Yet, especially in the case of fragile X syndrome, communication problems between the members of affected families have been documented (see Section 7.1.2), with the result that a discussion of these difficulties is warranted during the genetic counselling of the index case's parents (McConkie-Rosell et al., 1995).

For an index case who is a woman of childbearing potential with a family history of mental retardation, molecular testing now enables one to determine the genotypic status directly and to determine the risk of maternal transmission without having to gather as much information about the mentally retarded individual and his or her parents (Simonoff, 1998). This option can prove to be a definite advantage when the parents are not available, when communication about this matter is difficult between the relatives or when they are far away and have not kept in touch.

3.2.3 Genetic counselling of low-risk couples

Molecular tests are paving the way for the prospective screening of asymptomatic carriers, whose risk is, *a priori*, low. Currently, however, this approach has not been incorporated into clinical practice[46], and apart from a few research projects or pilot projects involving the screening of pregnant women, little experience has been reported in the literature with regard to this approach. Furthermore, there persist certain unresolved scientific, logistical and ethical problems (Fryns, 1995).

Nonetheless, if a premutation is detected in a woman who, *a priori*, is not at high risk, the purpose of genetic counselling would be to explain the nature of the syndrome and the impact of the test results for her and her family, and to determine the risk of transmitting the syndrome to her offspring[47]. However, practitioners must deal with the surprise and anxiety caused by the test results. In such circumstances, they cannot count on the benefits associated with making an accurate diagnosis in a symptomatic individual. Screening low-risk women does, however, have an important benefit, for it gives the couple the option of preventing the birth of an affected child, and the relatives can be informed of the existence of a high risk in their siblings. This specific contribution of molecular tests is all the more significant because families are smaller and smaller, because the expression of the syndrome is characterized by the phenomenon of anticipation and because the progressive nature of its manifestations makes it difficult for these couples to recognize a high risk in a timely fashion.

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[45] For example, a retrospective study from 1981 to 1992 covering three types of diagnostic tests (cytogenetic, linkage analysis and direct mutation analysis) available for genetic counselling showed that, during this period, more than one third of the carrier women (11 out of 27) saw their risk modified, which for some, meant undergoing a reversal of sterilization (Curtis et al., 1994). Ever since molecular diagnosis (direct mutation analysis) has been recognized as the gold standard (Oostra et al., 1993), the carrier status is clearly determined, which obviates the need to take drastic measures in situations of great uncertainty.

[46] The utility, feasibility and acceptability of asymptomatic carrier screening strategies will be examined in detail in Chapter 6 in order to determine the usefulness of promoting such strategies. For the time being, we are describing what, in theory, the contribution of molecular genetics to genetic counselling would be if it were provided in this context.

[47] The transmission risk assessment is based on data from families with at least one index case with the syndrome. The risk of transmission in the absence of a family history is being researched but has not yet been well-documented.

3.2.4 Residual problems

Although counselling can now, thanks to the developments in the molecular genetics of fragile X syndrome, rely on a better understanding of its mode of transmission and on the identification of the type of mutation, some problems do persist. They are due to an incomplete knowledge of the mutations' pathological mechanisms. These gaps in our knowledge basically have to do with a) predicting phenotype and b) assessing the risk of expansion of premutations during maternal hereditary transmission[48]. These gaps have an impact on genetic counselling, since they complicate the determination of the risk for the offspring and decision-making during prenatal diagnosis.

a) *Genotype/phenotype correlation and prognosis prediction*

As mentioned in Section 2.4, the main aspects of genotype/phenotype correlation are well established, but there are some unknowns in the following situations: in males with an allelic or methylation mosaic[49], who account for 15 to 20% of individuals with a full mutation, and in females with a full mutation, because of the variability in the clinical manifestations observed in them (Nussbaum and Ledbetter, 1995). Because of these gaps in our knowledge, it is especially difficult to predict these individuals' phenotype, as we do not presently have any validated tools[50] for making individual predictions. Future parents opting for prenatal diagnosis should be informed of the difficult decisions they might face in the event of an uncertain result.

The main difficulty concerns the decision facing couples when a prenatal diagnosis reveals a full mutation in a female fetus, since, in such cases, it is impossible to state with certainty if and to what extent there will be any mental retardation. The probability of the child developing at least mild mental retardation is nonetheless more than 50%[51]. These issues should be discussed before the tests are performed, so that the couple can make informed decisions at every stage of the consultation. Furthermore, support should be provided to couples faced with such difficult decisions, not only at the decision-making stage, but afterwards as well, whether their decision is to interrupt or continue with the pregnancy. In the latter case, the uncertainty as to the prognosis can persist for a long time because the symptoms appear gradually. This can have a chronic psychological effect on the parents.

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[48] The study of allelic stability during male meioses has, until now, been mainly of scientific interest (see Footnote 31 in Section 2.2), but this situation could change with the description of rare cases of father-daughter transmission of a full mutation (see Section 6.1.2 and Footnote 43 in Section 3.1).

[49] It will be recalled that individuals with an allelic or a methylation mosaic seem, on average, less severely affected from the standpoint of mental retardation and that some of them, high-functioning males, even have an IQ above 70.

[50] The measurement of the proportion of premutated alleles and the proportion of methylated mutated alleles are useful research tools for allelic and methylation mosaics, respectively, as is X chromosome inactivation ratio for females. However, studies examining the correlation with IQ have yielded contradictory results (Murray et al., 1997). As for the expression of FMRP, it has still not explained the entire clinical spectrum encountered in males with mosaics and in females with a full mutation (Kaufman et al., 1999).

[51] It should be borne in mind that, before molecular tests were available, similar decisions were made on the basis of a risk of mental retardation estimated at 30% for a female fetus.

b) *Allelic stability and risk of premutation expansion*

From data on trinucleotide expansions during mother-child transmission in fragile X families, one can assess, statistically and on a nonindividual basis, the probability of a given premutation expanding, based on its size. Since this risk is at least 10 to 20%, prenatal diagnosis is usually considered warranted, regardless of the size of the premutation. For grey zone alleles, there is no formal indication for prenatal diagnosis. Some authors nonetheless recommend studying the stability of these alleles in the family (Snow et al., 1993). A follow-up of certain families might be desirable, but this poses the problem of the long-term management of confidential and potentially anxiety-causing information.

The available data do not necessarily apply in the absence of a family history of mental retardation, but research is being carried out in this area. These data would be essential in the event consideration is given to screening pregnant women. Guidelines should then be adopted for women with a grey zone allele. In a pilot prenatal screening study in Finland, the women could undergo prenatal testing on request (Ryynänen et al., 1999).

3.2.5 Recap

The contribution of the molecular genetics of fragile X syndrome to genetic counselling is quite substantial. Molecular analysis of the FMR1 gene can be used to clearly establish a diagnosis of the syndrome in affected individuals, identify individuals at risk by confirming or ruling out the presence of a mutated gene in asymptomatic relatives, and determine the type of mutation, which, in turn, enables one to estimate the risk of transmitting the syndrome.

In families where fragile X syndrome has been clearly established and where the relatives are aware of it, molecular testing can reassure individuals who were experiencing anxiety over their family heredity and for whom the risk is negligible. Those who turn out to be carriers of a premutation or a full mutation can avail themselves of genetic counselling based on an assessment—which would be much more accurate than in the past—of the risk of transmitting the syndrome.

Lastly, since carrier status is determined on the basis of the genotype rather than the empirical risk determined from a pedigree analysis, pregnant women with a family history of mental retardation or of fragile X syndrome now have more accurate data for deciding whether or not to undergo prenatal diagnosis.

Nonetheless, a number of aspects of the pathological mechanisms of mutations have not been resolved, and this has an impact on genetic counselling and reproductive choices. All these uncertainties and the difficulties that result therefrom should be clearly explained to couples during genetic counselling so that they can base their decisions on sound, complete and up-to-date information.

3.3 DIAGNOSTIC INDICATIONS

Given that no clinical sign is pathognomonic for fragile X syndrome and given its variable clinical presentation, different indications for the molecular test can be considered. Since the symptoms become more evident with age, the use of narrow indications, such as morphological signs or mental retardation[52], lead to a later and less thorough diagnosis than the use of less suggestive but earlier signs, such as learning disabilities, language acquisition delay and behavioural problems. Besides, given the dynamic nature of the mutation, the disease occurs relatively often in the absence of a known family history of mental retardation[53]. A family history of mental retardation should therefore not be a necessary criterion for diagnostic testing. Otherwise, identification of the syndrome would be unduly limited.

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[52] Morphological signs and mental retardation are the clinical signs that were used to characterize the syndrome at the beginning, whereas the association with speech and

3.3.1 Guidelines

The indications for diagnostic testing are covered by guidelines (see Appendix III) issued by the American College of Medical Genetics (ACMG, 1994) and the American College of Obstetricians and Gynecologists (ACOG, 1996). Both organizations state that any child with developmental delay and any individual with mental retardation of unknown etiology should be referred for a genetic workup that includes karyotyping[54] and molecular testing for fragile X syndrome. The ACMG stresses that these indications are all the more applicable when there are suggestive morphological signs or a family history of fragile X syndrome or mental retardation. Additionally, the ACMG, but not the ACOG, also recommends testing to rule out fragile X syndrome in any child with signs of autism.

3.3.2 The diagnostic workup for developmental delay or mental retardation

In the recent literature, various types of studies have shown the deficiencies in the diagnostic workup of developmentally delayed or mentally retarded children (Magnay et al., 1996; Gringras et al., 1998). Several authors and committees have examined the more appropriate diagnostic protocols. They point out that, with the most thorough workups, a much higher proportion of developmentally delayed or mentally retarded children could be etiologically diagnosed[55], which leads most often to more accurate genetic counselling for the family (Curry et al., 1997; Majnemar and Shevell, 1995; Battaglia et al., 1999; Strømme and Hagberg, 2000). A consensus conference organized by the American College of Medical Genetics (October 1995) on the clinical and diagnostic workup of patients with mental retardation concluded that, if there is no known etiology, molecular analysis for ruling out fragile X syndrome is indicated in most cases (Curry et al., 1997). In the context of clinical services provided on the request of families[56], it would be logical for fragile X testing to be part of these efforts to improve and standardize the diagnostic approach for developmental delay and mental retardation.

Since mental retardation involves not only cognitive functional impairment, but also results in limitations in the affected individual's adaptive functioning in his or her social environment[57], an etiological workup should be carried out, during childhood, with an exhaustive assessment of the individual's cognitive and adaptive skills[58]. Such an assessment is essential and should be revised periodically, if one wishes to individualize the social and educational

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behavioural problems was described only later and has been the subject of fewer studies.

[53] The authors of two clinical series report that about 35% of the affected individuals did not have a known family history of mental retardation (Mornet and Simon-Bouy, 1996; Cossée et al., 1997). However, this proportion depends on the indications chosen by those who order the test.

[54] We are referring here to the usual karyotyping, not the specific cytogenetic test for detecting fragile sites. Several studies have shown that chromosomal abnormalities are as frequent, if not more frequent, than fragile X syndrome in children referred for the fragile X test (Hagerman et al., 1988; Voullaire et al., 1989; Turner et al., 1992; Dewald et al., 1992; Jenkins et al., 1992; van den Ouweland et al., 1994; Howard-Peebles et al., 1995; Marini et al., 1997).

[55] Nonetheless, in 20 to 35% of children, an etiologic diagnosis cannot be made, despite an exhaustive workup.

[56] Excluding more proactive diagnosis and screening strategies.

[57] See Section 1.3.1.

[58] For a discussion of the concept of adaptive skills and their measurement, see Dykens (1995).

social and educational interventions by adjusting them to each person's strengths and weaknesses.

3.3.3 Broadening the indications

Given the insidious onset and the variability of the syndrome's first outward signs, it would certainly not be desirable that the molecular test be restricted to the sole indications of mental retardation and developmental delay. The purpose of broadening the indications to children with less suggestive clinical signs, such as language delay, attention disorders, autistic tendencies or learning disabilities[59] is to make an earlier diagnosis (so that the families can avail themselves of the necessary services sooner) and to identify a larger proportion of affected individuals (so that more families can obtain genetic counselling).

However, relatively few studies are convincingly in support of systematically testing children with these isolated (i.e., in the absence of mental retardation) clinical signs (see Sections 6.1.1. and 6.1.3). It would be desirable for other studies to continue assessing the yield of molecular analysis for these broadened indications and for professional associations to adjust their recommendations in light of the results. While it is acknowledged that the usefulness of diagnostic testing can be examined on a case-by-case basis in a clinical practice setting, taking the entire clinical picture into account, the use of proactive strategies of diagnosis and carrier screening based on broadened indications cannot be considered in the absence of a more thorough assessment.

By broadening the diagnostic indications, the total number of tests ordered and the proportion of negative tests could increase, which can pose a logistical problem, since the validated tests are unsuitable for wide-scale use. These logistical problems and the desire to limit the number of unnecessary tests have led certain researchers to enrich their samples by prescreening, using additional clinical criteria in the form of checklists. However, there is no general agreement as to the use of these prescreening criteria.

3.3.4 Prescreening checklists

Several authors have proposed checklists based on 5 to 17 physical and behavioural criteria for use as screening tools in order to reduce the number of tests performed and to improve the yield (positivity rate) of proactive screening strategies (Hagerman et al., 1991; Butler et al., 1991; Laing et al., 1991; Giangreco et al., 1996; Hećimović et al., 1998; de Vries, 1999a). The criteria used vary from one instrument to another, which is probably due to the method used to select the individuals who took part in validating the checklists and especially to their age. For example, physical characteristics, such as macroorchidism[60] and large, prominent ears, are two of the criteria used most often in checklists tested on a mainly adult population but have little chance of being as discriminating when applied to a prepubertal population (Mandel et al., 1994). To overcome the problem of screening children, it was recently recommended that behavioural characteristics be included[61] (Mazzocco, 2000).

Often, these checklists have not been completely validated, since those developed from a retrospective study (Giangreco et al., 1996; Hećimović et al., 1998) or those resulting from the secondary selection of the most significant items (Butler et al., 1991; Giangreco et al., 1996; Teisl et al., 1999) would need to be reevaluated prospectively. Some checklists have been evaluated only in boys (Hagerman et al., 1991; Butler et al., 1991; de Vries, 1999a). Several authors conclude that these checklists are useful in that they result in fewer tests being performed and a higher positivity rate[62], although their performance is not always adequately described[63]. When all is said and done, there seems to be no general agreement on any one checklist at the present time. Researchers who are instituting proactive screening strategies continue to adjust the existing checklists to their own respective populations. Also, their clinical use is very limited.

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[59] The definition of learning disabilities poses a problem, which we discuss in Section 6.1.3.

[60] Sabaratnam et al. (1994) raise the issue of the acceptability of performing certain physical measurements (testicle size, head circumference, ear size) in the context of prescreening that does not necessarily involve a complete physical examination by medical personnel in a separate examining room.

[61] Teisl et al. (1999) propose a checklist based solely on behavioural characteristics for use in tandem with another checklist for the purpose of identifying school-age children who might be less symptomatic.

3.3.5 Premutations and early ovarian failure

Other strategies are being developed to identify at-risk families, especially those without any affected children. In the early 1990s, it was hypothesized that there is an association between positive fragile X carrier status and early or premature ovarian failure[64] (Cronister et al., 1991). Subsequently, several studies, including some multicentre ones, provided arguments in support of this hypothesis (Schwartz et al., 1994; Conway et al., 1998; Murray et al., 1998; Giovannucci-Uzielli et al., 1999; Marozzi et al., 2000), whereas others tended to refute it (Kenneson et al., 1997). Quite recently, the preliminary results of an international, multicentre study confirmed the existence of an association between premature ovarian failure and positive premutation carrier status[65] (Allingham-Hawkins et al., 1999). Depending on their design, these studies documented the proportion of women carriers who experienced early or premature ovarian failure[66], or the proportion of women who experienced early or premature ovarian failure and who were premutation carriers[67]. However, the mechanism underlying this association has yet to be elucidated[68].

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[62] Laing et al. (1991) used their 5-item checklist on a large number of individuals in an Australian screening program. They report a reduction in the proportion of eligible individuals tested from 82 to 47%, with the positivity rate increasing from 4.43 to 9.13%, for a stable prevalence of the syndrome in individuals with mental retardation. Giangreco et al. (1996) report a 60% reduction in the number of tests performed and a 9.5% positivity rate. De Vries et al. (1999a) observed an 86% decrease in the number of tests and an eightfold increase in the positivity rate.

[63] Performance can be determined from data published in certain cases: sensitivity = 86.7%, specificity = 84.7%, positive predictive value = 48.2%, negative predictive value = 97.5% for a result greater than or equal to 16 on Hagerman et al.'s 13-item checklist (1991); sensitivity = 47%, specificity = 98.3%, positive predictive value = 75%, negative predictive value = 94% for a result greater than or equal to 21 on Butler et al.'s 15-item checklist (1991); sensitivity = 100%, specificity = 60%, positive predictive value = 8.5%, negative predictive value = 100% for a result greater than or equal to 5 on Giangreco et al.'s 6-item checklist (1996); sensitivity = 100%, specificity = 87% for de Vries et al.'s 7-item checklist (1999a). Laing et al. (1991) and Teisl et al. (1999) do not present their data in a way that the performance of their checklists can be correctly determined, and Sabaratnam et al. (1994) and Arvio et al. (1997), who recommend adjusting checklists to the population of interest, do not do an actual performance assessment.

[64] Early ovarian failure, which is defined as menopause before the age of 47, occurs in approximately 10% of women. Premature ovarian failure, which occurs before the age of 40, is experienced by only about 1% of women. In both cases, more than a third of the women in question are reported to have a family history.

[65] Schwartz et al. (1994) suggested that, unlike women with the premutation, those with a full mutation are not at increased risk for early or premature ovarian failure. This was confirmed by subsequent studies.

[66] This proportion varies according to the age group considered and the exclusion criteria: 20 to 25% of the 92 women with a premutation in Schwartz et al.'s study (1994); 18.8% of the 170 women over the age of 18 with a premutation in Giovannucci-Uzielli et al.'s study (1999); and, in an international, collaborative study (Allingham-Hawkins et al., 1999), 16% of the 128 women over the age 18 with a premutation, but 23% of those over the age of 40.

[67] Kenneson et al.'s study (1997) involved 216 women with early ovarian failure but only 33 with premature ovarian failure, among whom none turned out to be premutation carriers. However, Murray et al. (1998) documented a premutation in 4% of the 147 women with premature ovarian failure tested, and Giovannucci-Uzielli et al. (1999) found a premutation in 6.48% of the 108 women with premature ovarian failure. Marozzi et al. (2000) found a premutation in 6% of the 106 women with premature ovar-

Since the prevalence of the premutation in women with idiopathic premature ovarian failure is much greater than that in the general population, most authors (Conway et al., 1998; Murray et al., 1998; McPherson et al., 1999; Giovannucci-Uzielli et al., 1999; Marozzi et al., 2000) recommend fragile X screening of this at-risk population in order to identify at-risk families before a first child is born[69]. Furthermore, information about this association should be provided to all women in whom a premutation has been found, given the impact that it can have on their reproductive decisions and on the timely management of the medical follow-up of menopause (Allingham-Hawkins et al., 1999).

3.3.6 Recap

Developmental delay and mental retardation of unknown etiology are formal diagnostic indications for fragile X molecular testing. Ideally, in the context of clinical services provided on the request of families, the testing should be part of an etiologic workup for developmental delay and mental retardation, which is presently being standardized and which should include, among other things, a genetic and neurological workup, an exhaustive assessment of the individual's cognitive and adaptive skills, and karyotyping.

Broadening the indications to children with less suggestive clinical signs should be considered if one wishes to make the diagnosis earlier so that the families can avail themselves of the necessary services sooner and to identify a larger proportion of affected individuals so that more families can obtain genetic counselling. However, relatively few studies are convincingly in support of systematically testing children with speech delay, attention disorders, autistic tendencies or learning disabilities in the absence of mental retardation. It would therefore be desirable for studies assessing the yield of molecular testing for these broadened indications to continue and for the professional associations to adjust their recommendations in light of the results.

Given this broadening of the indications and in order to limit the number of tests, some investigators have used prescreening criteria, especially in the context of proactive diagnostic and screening strategies. However, the validation of these checklists is incomplete, and there seems to be no general agreement on any one checklist at this time.

Lastly, the existence of an association between premature ovarian failure and positive premutation carrier status was recently documented, with the result that screening this high-risk population is now an additional means of identifying affected families.

3.4 MOLECULAR DIAGNOSTIC PROTOCOLS

Since the dynamic mutation is the main one encountered in fragile X syndrome, genotypic analysis basically consists in determining the size of the trinucleotide expansion and the methylation status of the FMR1 gene. In very rare cases where strong clinical presumption of fragile X syndrome is not corroborated by the results of the molecular analysis of the trinucleotide repeats, it may be advisable to search for an expansion at the FRAXE fragile site or for point mutations or deletions in the FMR1 gene. How-

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ian failure. The association might be stronger in cases of a family history of premature ovarian failure (Conway et al., 1998; Murray et al., 1998; Marozzi et al., 2000).

[68] It cannot be reduced FMRP synthesis because the association concerns premutations but not full mutations.

[69] Conway et al. (1998) also point out that, since women with early ovarian failure will sometimes accept an egg donated by a family member, the fact that several relatives might have a premutation should be brought up during genetic counselling.

ever, this step is performed in a research setting[70] and will not be mentioned in the description of the protocol provided below, which pertains exclusively to the available clinical services.

Even if a single mutation may be searched for, the analysis of the trinucleotide expansion is relatively complex because of the wide array of alleles found in affected individuals. No one method lends itself perfectly to the detection of all these alleles: the Southern method is best suited for detecting large expansions, while selective *in vitro* DNA amplification, or polymerase chain reaction (PCR), permits an accurate determination of the size of normal alleles and small expansions. These two techniques are therefore generally used together. We present below the reference method for diagnosing the syndrome and the main alternative approaches. The reference method is based on the use of the Southern technique followed, if necessary, by PCR, while the main alternative approach uses PCR, supplemented by the Southern technique, if need be. Several protocols have been proposed for this latter approach. They are described in Appendix II, and their validity is discussed in Chapter 4 and Appendix II.

3.4.1 The reference method

Genotypic analysis for fragile X syndrome is based on the use of two techniques, Southern blot and PCR, which are used sequentially (Oostra et al., 1993; Snow et al., 1993). The protocol used most widely for the Southern method is that described by Rousseau (Rousseau et al., 1991a, 1992), which involves the use of two restriction enzymes, EcoRI and EagI. The probe StB12.3 detects a 5.2-kb fragment resulting from cleavage by the enzyme EcoRI if the CpG island is methylated and a 2.8-kb fragment resulting from double digestion (EcoRI and EagI) if there is no methylation. With this double enzyme digestion, one can therefore estimate the size of the expansion and determine the gene's methylation status, which helps distinguish between full mutations, which are methylated, and premutations, which are unmethylated. Figure 3.1 (on the next page) is a schematic representation of the results of the Southern method for the simplest scenarios (Rousseau, 1991b).

The Southern method can detect alleles with between about 70 (50 at laboratories with the most expertise) and several thousand CGG triplets. However, at the lower limit of this range, discrimination between alleles is difficult. Yet an accurate estimate of the number of triplets in them is especially important for distinguishing between normal alleles and small premutations. To overcome this problem, attempts have been made to adapt PCR to the diagnosing of fragile X syndrome.

With PCR, one can easily amplify normal alleles and small premutations by means of probes flanking the trinucleotide repeat. The protocol originally proposed by Fu et al. (1991) is based on the visualization of radioactive amplification products after polyacrylamide gel electrophoresis, which permits an accurate determination of the number of repeats in these alleles. One can therefore clearly distinguish between normal alleles and small premutations, thus remedying the deficiencies of the Southern method in this regard.

Figure 3.1: Schematic representation of the hybridization profiles obtained for fragile X syndrome by the Southern method (EcoRI + EagI double enzyme digestion) in the absence of a mutation and in the presence of a premutation, a full mutation and an allelic mosaic.

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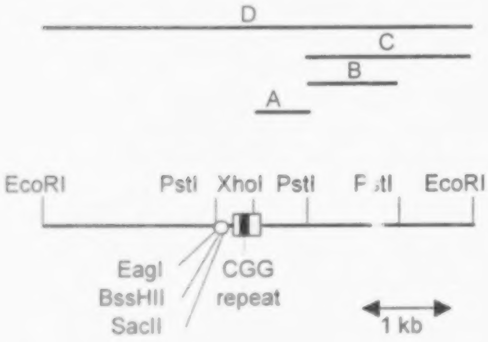
[70] Given the limited number of cases, it does not seem necessary, at the Canadian level, to have more than one research laboratory devoted to such analyses.



Source: Rousseau et al. (1991b)

Legend: With a normal number of CGG triplets, only the 2.8-kb fragment is visible in males (M), while both fragments (2.8 kb and 5.2 kb) are observed in females (F), since the X chromosome is present in both an active, unmethylated form and an inactive, methylated form. A premutation also occurs in a methylated and unmethylated form in females, but only in an unmethylated form in males. Full mutations are generally methylated and often appear as a smear because of their mitotic instability.

The reference method for diagnostic analyses therefore consists of the Southern method followed by PCR, if the observed allele is at the upper limit of normal.

3.4.2 The alternative methods

Since the reference method is relatively labour-intensive and difficult to apply on a large scale, many researchers have attempted to develop methods in which samples would first be screened by PCR, even if it means subsequently confirming doubtful or abnormal results by the Southern method. However, amplifying large expansions, which have a particularly high cytosine and guanine content, is difficult. Many technical variants of PCR have been tried out in order to facilitate the amplification of large expansions. These variants, which are described in Appendix II, differ in their amplification (enzymes, probes, etc.), amplimer separation and amplimer visualization (radioactive, chemiluminescent, fluorescent or other methods) techniques. Although most of these methods are faster[71] and less expensive than the Southern method, they remain, in general, relatively complex, as some require, for example, transferring the amplification products onto a membrane, followed by hybridization.

Furthermore, these methods, which use PCR first, cannot be used alone, since some full mutations escape amplification[72] and since confirmation by the Southern method is necessary for a sizable proportion of samples. In males, nonamplification suggests the presence of a mutation, while in females, the visualization of a single allele can be due to failure to amplify a full mutation but can also indicate the presence of two identical or almost identical alleles, since 20% of normal females are homozygous for an allele of less than 54 triplets[73] (Brown et al., 1996). Consequently, when no allele is visualized in a male or when a single allele is detected in a female, diagnostic confirmation must necessarily be obtained by the Southern method. Lastly, the Southern method is required for determining the methylation status of the FMR1 gene and therefore for differentiating large premutations from small full mutations.

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[71] Not only is PCR faster, but it also requires only a trace amount of DNA, which permits a direct analysis (without prior cell culture) on an amniocentesis specimen, on a buccal smear or on a specimen from a Guthrie card. Several authors have taken advantage of this to carry out screening in schools using buccal smears (Hagerman, 1994b; Meadows et al., 1996; Crawford et al., 1999; Murray et al., 1996).

[72] Up to 10% of full mutations, according to Brown (1996) and Brown et al. (1996), but this figure most likely varies according to the technical variant.

[73] Brown et al. (1996) estimated at 20% the expected homozygosity rate according to the prevalence of the different alleles detected in 2,500 X chromosomes without mutations in the FMR1 gene. However, the homozygosity rates actually observed ranged from about 20 to 35% (Murray et al., 1997) as they depend on the technique and experimental conditions.

Despite these precautions, the nonamplification of a full mutation may cause false-negative results in a number of circumstances. In females or in the presence of an allelic mosaic in males, the preferential amplification of shorter alleles can result in an incorrect diagnosis (Erster et al., 1992; Snow et al., 1993; Brown et al., 1993; Haddad et al., 1996). This situation is especially problematic in the prenatal diagnosis of female fetuses (Abd-El-Aleem et al., 1995; Brown et al., 1996). For these reasons, some researchers recommend using PCR first only in males and confirming the results with the Southern method, even if a premutation has been identified[74]. Beyond the specific indications for PCR, which are the subject of debate, the performance of this approach has not been adequately documented for use on a first-recourse basis (see Section 4.2 and Appendix II for a more detailed discussion). It must be recognized that, despite these problems, PCR on a first-recourse basis is an approach that is being utilized more and more (Holden et al., 1995a, 1995b; Brown et al., 1996; Meadows et al., 1996; Murray et al., 1996; de Vries et al., 1997; Gérard et al., 1997; Hećimović et al., 1998; Crawford et al., 1999; Ryynänen et al., 1999; Millan et al., 1999; Youings et al., 2000).

A totally different approach, which is based on the study of the gene's expression by immunocytochemical analysis of FMRP on blood smears or hair roots, has also been tried out (Willemsen et al., 1995, 1997b, 1999). The blood smear technique detects full mutations in males but not premutations, which can be beneficial in certain screening strategies, but the discriminating power of this approach might not be as good as expected (de Vries et al., 1998c; Tassone et al., 1999). Although it is faster than the Southern method, it requires a microscopic examination of a large number of lymphocytes. The hair root technique is fast and can reportedly be used in males and females, but it still needs to be validated.

Many research teams are presently continuing their work to improve the existing techniques or to develop others that are faster, less labour-intensive and less expensive than the conventional Southern method while at the same time avoiding the pitfalls still encountered in the current variants.

In short, we can expect rapid developments in this field, but a rigorous comparison of these new methods with the reference method should be carried out before they are incorporated into clinical services, in order to assess the risks and benefits associated with them. It could be that, in the future, different approaches will be used according to the diagnostic or screening strategy adopted, in particular, according to the target population. Until they are validated, the reference method is still the Southern method followed by PCR, if necessary.

3.4.3 Prenatal diagnosis

Prenatal diagnosis raises more problems than diagnosing individuals who are potentially affected or who are potential carriers. Not only do the specimen collection methods—chorionic biopsy or amniocentesis—involve risks, but performing and interpreting the tests per se is also a more complex task. On the one hand, visualizing full mutations following amniocentesis can be difficult in female fetuses if the Southern protocol (Rousseau et al., 1991a) is used alone. To get around this problem, it is advisable to perform a second enzyme digestion, with the enzyme BglII, for example, in order to obtain more condensed hybridization bands. On the other, methylation of full mutations and inactive X chromosomes is not always completed in extraembryonic tissues by around the 10th week of pregnancy (Sutherland et al., 1991; Oberlé et al., 1991; Castellví-Bel et al., 1995). Yet, chorionic biopsies are performed between the 10th and 12th week. As a result, in the case of the chorionic biopsy, it is sometimes difficult to distinguish between large premutations and small full mutations[75], interpreting mosaics is problematic[76], and ambiguous hybridization profiles are sometimes observed even with full mutations. Errors are also possible because of maternal contamination[77] (Maddalena et al., 1994). Furthermore, the gender of the fetus must be determined by other methods (e.g., Y chromosome-specific PCR).

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[74] This, more restricted protocol could still yield erroneous results in cases of allelic mosaics including normal alleles and full mutations, even if this situation seems rather rare (Snow et al., 1993; van den Ouweland et al., 1994; Rousseau et al., 1994; Nolin et al., 1994; Haddad et al., 1996; Perroni et al., 1996; Mingroni-Netto et al., 1996; Milà, 1996; Orrico et al., 1998; Schmucker and Seidel, 1999; Gold et al., 2000).

PCR is not indicated as a first recourse, since visualizing full mutations in female fetuses is also a problem with this method. However, PCR can sometimes be used to clarify an ambiguous situation after Southern analysis (Castellví-Bel et al., 1995). In addition, the interpretation of prenatal diagnosis results can be facilitated by knowing which alleles are carried by both parents, with the result that it is preferable to accurately determine the size of the alleles in the child and the parents.

In conclusion, several authors use both methods, Southern and PCR, on a routine basis for all prenatal diagnoses (Castellví-Bel et al., 1995; Brown et al., 1996). As regards chorionic biopsy, caution should be exercised, since the results can be ambiguous because of differences in methylation between the embryonic and extraembryonic tissues[78]. In inconclusive cases[79], amniocentesis should be performed after the chorionic biopsy. Since two-stage prenatal screening is not necessarily accepted, it is of the utmost importance to inform couples undergoing a chorionic biopsy of the possibility of inconclusive results.

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[75] It is advisable to check the methylation status by amniocentesis if the chorionic biopsy reveals an expansion of 400 to 700 bp (Rousseau et al., 1991a).

[76] In 1993, Oostra et al. (1993) went as far as recommending confirming any premutation detected upon chorionic biopsy by amniocentesis, but it does not seem that this recommendation is being followed.

[77] Maddalena et al. (1994) reported three cases of maternal contamination in 22 chorionic biopsies.

[78] The recommendations of professional associations in the United States differ in this regard. The American College of Medical Genetics underscores this warning but does not proscribe chorionic biopsy (ACMG, 1994), while the American College of Obstetricians and Gynecologists recommends only amniocentesis for prenatal screening but does not mention the option of chorionic biopsy (ACOG, 1996).

[79] To our knowledge, no large study has accurately documented the proportion of chorionic biopsies whose results are inconclusive. Grasso et al. (1996) report that they performed an amniocentesis because of inconclusive results in three cases in 22 chorionic biopsies (12%). Maddalena et al. (1994) report one ambiguous case in 22, in addition to the three cases of maternal contamination (18% in all). It should be noted that amniocentesis sometimes yields inconclusive results as well, as demonstrated by the Québec experience (see Section 5.2).

4. THE PERFORMANCE OF MOLECULAR TESTS

The evaluation of molecular genetic tests poses—perhaps more acutely than for other tests—the problem of choosing a suitable reference test, or "gold standard". And there are two types of validation, depending on whether the phenotype of the disease or the genotype is considered to be the reference. One speaks of clinical or diagnostic validity in the former case and of analytical validity in the latter.

In certain diseases, the clinical signs and the results of conventional diagnostic tests suffice to classify individuals being diagnosed as having or not having the disease and thus to serve as a reference for the clinical validity of the applicable molecular tests. However, a definite diagnosis cannot always be made on the basis of the clinical findings. Molecular genetic tests are becoming reference tools, even often serving to reconceptualize clinical entities (the genetic nosology supplanting the clinical nosology). Such is, among others, the case with fragile X syndrome, whose symptoms vary and in which no clinical sign is pathognomonic. Furthermore, the performance of cytogenetic analysis, the only diagnostic approach previously available, was seriously called into question after the development of molecular tests.

As for analytical validity, no one method alone seems to be an absolute reference tool for the genotype. Therefore, new methods are usually compared to the older ones. In the case of fragile X syndrome, the techniques that contributed to the identification of the dynamic mutation and the gene, especially the Southern blot method, still constitute the basis of the analytical protocols for diagnostic purposes. These techniques and those developed subsequently are detailed in Appendix II.

In general, both in research and in routine practice, each technique is accompanied by a certain number of internal controls that help minimize the error rate. Some controls are run when preparing the reagents, while others are performed with each reaction. It is of the utmost importance to check the concordance of the results obtained for each member of a given family with the pedigree data and, if applicable, to check, for each individual, the concordance between the different techniques used.

4.1 CLINICAL VALIDITY

The dynamic mutation is by far the mutation described most often in fragile X syndrome. Rare point mutations have been identified as well in the FMR1 gene (De Boulle et al., 1993; Lungenbeel et al., 1995), as have a few full or partial deletions. In 1997, Hammond et al. (1997) identified 24 families in whom a deletion had been documented. Since then, a few other cases have been reported in the literature (Petek et al., 1999; Parvari et al., 1999; Garciá Arocena et al., 2000). However, these families constitute a minority of fragile X families, probably less than 2%. Therefore, for a method that detects all expansions, the theoretical clinical sensitivity for diagnosing affected individuals could be estimated at 98%. However, a more rigorous estimate of the clinical sensitivity should be based on a study involving a more systematic search for point mutations and deletions when a dynamic mutation is not found. When a dynamic mutation has already been documented in a given family and when the family is screened for carriers of this mutation, the problem of clinical sensitivity does not apply[80].

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[80] Thus far, no new mutation has been documented in a family with an index case.

4.2 ANALYTICAL VALIDITY

Determining the analytical validity of a molecular test consists in checking if all the carriers of an expansion (true positives) can be identified, whether the expansion is small or large, and, conversely, that the test does not indicate the presence of a mutated allele in individuals with two normal alleles (false positives).

Initially, the Southern method was compared with **cytogenetic analysis**. However, the Southern method quickly supplanted cytogenetic analysis as the reference tool, given that discrepancies were attributed to errors in cytogenetic analysis. The latter yielded numerous false positive results because of the existence of fragile sites near the FRAXA site. From this standpoint, molecular genetics helped make a distinction between two causes of mental retardation (associated with the FRAXA and FRAXE fragile sites) and therefore helped reconceptualize the very clinical entity. Conversely, many negative results with cytogenetics actually involved small expansions detectable by the Southern method and were therefore false negatives. These diagnostic errors attributable to cytogenetic analysis occurred both during the diagnosing of potentially affected individuals and during prenatal diagnosis (Rousseau et al., 1994; Jenkins et al., 1995; Morton et al., 1997).

After 1993, the **Southern method** replaced cytogenetic analysis for diagnosing fragile X syndrome (Oostra et al., 1993). The protocol most widely used involves double enzyme digestion, which enables one to determine the size of the alleles and to determine the gene's methylation status (Rousseau et al., 1991a). This method permits visualization of a large array of alleles but not a fine resolution of the number of repeats. The larger the trinucleotide expansion, the more difficult it is to estimate its size because of somatic mosaics, which appear as multiple hybridization bands or as a smear. For full mutations, the estimates made on a given gel by different expert observers vary most often from about 250 to 500 bp, but differences greater than 1 kb have been observed as well (Fisch et al., 1996a). These differences have apparently not led to any classification errors between premutations and full mutations (Fisch et al., 1996a). In any case, it is the methylation status that is used to make this distinction.

In contrast, distinguishing between premutations and normal alleles is more difficult, and false negatives could occur, depending on the laboratory's expertise (with regard to gel resolution and interpretation) if only the Southern method with the enzymes EcoRI and EagI is used (Fisch, 1996a). Therefore, when an allele seems to be at the upper limit of normal, one must complete the analysis by performing PCR or an additional digestion using PstI enzymes, which yields smaller fragments. The PCR method, with electrophoretic separation on polyacrylamide gel, seems to be the approach generally preferred (Snow et al., 1993), since it can, in optimal conditions, distinguish between alleles that differ by only a single trinucleotide repeat. Lastly, the possibility of false-positive results associated with incomplete digestion by the enzyme EcoRI has been raised, with the result that some researchers prefer to use the enzymes HindIII and EagI (Storm et al., 1998).

In short, even if the Southern method is considered the method of choice for diagnosing fragile X syndrome, the existence of artifacts that can lead to confusion and of interobserver differences in determining the size of alleles underscores the expertise required for interpreting the results. As for the carrying out of this type of analysis, it also depends on precise experimental conditions, with the result that test performance can vary from laboratory to laboratory.

The PCR method used as a backup technique to the Southern method for determining allele size and distinguishing between normal alleles and small premutations is generally inspired by the method developed by Fu et al. (1991) for studying the stability of these alleles. With polyacrylamide gel, one can, in optimal experimental conditions, separate two alleles that differ by a single triplet, which is extremely important for distinguishing between normal alleles and premutations. Indeed, while a classification error that results in a false-negative diagnosis would probably have little consequence for the individual's prognosis, it would give him or her false reassurance as to the risks for his or her offspring, who could actually carry larger expansions.

Numerous **variants of the PCR method** designed to replace the Southern method as the method of first recourse have been developed in order to facilitate the wide-scale use of the molecular testing. Although these alternatives have scored some points in relation to the original method with regard to the amplification of large expansions, which is difficult to achieve, or the separation or visualization of amplification fragments, most authors agree that, whatever variant is considered, there persists a major problem due to the fact that the preferential amplification of small alleles can compromise the identification of full mutations. The problem cases are essentially allelic mosaics in males, which can be mistaken for premutations (Erster et al., 1992; Snow et al., 1993; Brown et al., 1993; Haddad et al., 1996), and full mutations in female fetuses, in whom false negatives have also occurred (Abd-El-Aleem et al., 1995; Brown et al., 1996). The few reported false positives resulted from maternal contamination during prenatal diagnosis by chorionic biopsy. Lastly, many researchers are attempting to determine the gene's methylation status by PCR, but these methods are still in the research stage.

Although a number of researchers have done a limited comparison of their techniques with Southern blot, the validation of these techniques is generally less than satisfactory (see details in Appendix II). In particular, the error rate to be expected should be determined on the basis of a systematic, comparative study with the reference method (Levinson et al., 1994). There are substantial differences between the various published protocols, and it is routine practice for molecular genetics laboratories to make various modifications to the analytical protocols and experimental conditions. Yet, to our knowledge, no rigorous, independent, peer-reviewed, comparative study has been carried out between the different techniques or between them and the Southern method in order to assess their validity in the context of diagnostic services rather than in the context of research tools.

Furthermore, whether classification errors occur depends not only on the tests' technical performance, which is particularly sensitive to the experimental conditions, but also on the interpretation of the test results and on the control procedures that follow, in this case, accurate confirmation of the results by the Southern method. Yet, there is no consensus in the literature as to the precise indications for using PCR first or as to the circumstances in which the results should be checked by the Southern method.

Apart from their validity, which still needs to be documented, instituting such tests in a clinical laboratory requires the necessary expertise for developing the technique and ensuring, on a local basis, its quality (calibration, performance checks, reproducibility, etc.), the expertise for interpreting the results, as well as taking part in an external control process on a periodic basis.

In conclusion, the Southern method, which contributed to the identification of the dynamic mutation and the gene, is still used for diagnostic purposes. It is used on a first-recourse basis and is followed, if need be, by PCR in order to accurately determine the size of normal alleles and small expansions. This sequential-type protocol constitutes the reference method for diagnosing and screening for the syndrome and permits genotypic diagnosis of the vast majority of the individuals concerned.

A number of researchers recommend protocols in which PCR is performed first, with selective confirmation by Southern blot. Worldwide, a few clinical laboratories have adopted this approach. However, there is no consensus in the literature as to the optimal protocol or to the precise indications for using such protocols. Furthermore, no rigorous, systematic study of the analytical validity of these techniques (in relation to the Southern method) has been carried out, which should be done before instituting them in a clinical setting.

Instituting quality controls more systematically would also be desirable, for test performance could vary with the experimental conditions, even if numerous technical precautions and internal control mechanisms are developed to minimize the risk of errors. The expertise required for interpreting the results underscores the importance of close collaboration between research laboratories and clinical laboratories.

5. THE SITUATION IN QUÉBEC

In this chapter, we review the manner in which a vast range of medical, social and educational services used by fragile X individuals and their families are organized. For each sector, we attempt to determine if the available services adequately meet the specific needs of fragile X children and their families. The evaluation is however limited by the fact that the offer of services is organized according to many different classifications. Yet, these children's needs do not necessarily fit with these classifications. For example, while most of the needs generally result from mental retardation, language acquisition problems, learning disabilities, and behavioural and socialization problems create additional needs, which must be factored into the offer of services.

This description of the situation in Québec is a prerequisite for evaluating the diagnostic and screening strategies presented in the next chapter. While their feasibility depends, of course, on the regional context, their usefulness, too, can be assessed only in light of the services presently provided to families. First, before describing the various services, we present the available data on individuals who may have the syndrome and identify the limits of these data with a view to discussing the different strategies.

5.1 PREVALENCE

In order to assess the extent of the problem in Québec and to document the number of affected individuals, affected families and at-risk individuals, we examine below both statistics from elsewhere and the empirical data available for Québec.

Table 5.1 provides a theoretical estimate of the number of fragile X individuals in Québec based on the latest prevalence data on the syndrome. Assuming a prevalence of 1 per 4,000 boys and 1 per 8,000 girls, and based on Statistics Canada data for the Québec population in 2000, we estimate, for example, the number of children aged 0 to 14 years who have the syndrome to be 250, for a total of 1,377 affected individuals. As mentioned earlier, the data that we have probably provide a minimum prevalence, given the method of identifying affected individuals in the studies on which these figures are based.

Based on this prevalence figure and on the number of births in Québec in 1999-2000, we estimate that about 9 or 10 affected boys and 5 affected girls were born that year, with the number of children who will develop mental retardation being 735, assuming a minimum prevalence of 1% for mental retardation. In this scenario, fragile X syndrome is responsible for 1.9 to 2% of the cases of mental retardation[81]. Actually, the prevalence of mental retardation is poorly known. It reportedly occurs in 1 to 3% of the population (Curry, 1997; Roeleveld, 1997), which means that the number of affected children in this birth cohort could be as high as 2,205, thus reducing proportionately the percentage attributable to fragile X syndrome.

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[81] This proportion is consistent with the figure of 1.8 to 2% reported by Turner et al. (1986, 1992).

Table 5.1: Theoretical estimate of the number of fragile X individuals, by age and sex, in Québec in 2000

Age group	Males	Females	Total
0-14 years	169	81	250
15-64 years	644	317	961
65 years and +	97	69	166
Total	910	467	1377

The number of children in whom developmental delay or language acquisition delay is observed during the first five years is even more difficult to estimate. The number of medical visits for which these two diagnoses are reported cannot constitute a valid estimate of the incidence of these two problems and can, at best, provide a very approximate indication of the services provided to this population[82]. Thus, between 1994 and 1999, on average, there were 1,000 medical visits per year for developmental delay in children under the age of 5 years, excluding services obtained in hospitals. For language acquisition delay, the number of visits rose sharply during this period, from 1,400 in 1994-1995 to 2,200 in 1998-1999. Furthermore, no data are available on the number of visits to psychologists or speech therapists.

In countries like Australia and the Netherlands, where, as part of programs or research projects, there has been more-systematic fragile X case-finding in institutions for the intellectually impaired or in special schools, this hereditary condition is still underdiagnosed (see Section 6.1.3 for more details). The extent of this underdiagnosis depends on the resources dedicated to ensure diagnosis of affected individuals and follow-up of their families, on the duration of the program and on the target population's participation in it. In Australia, about 75% of the affected males have been diagnosed (Turner et al., 1997), while in the Netherlands, this figure is thought to be less than 50% (de Vries et al., 1997, 1998d). It is very likely that, in areas where such programs do not exist and where affected families have been identified only via clinical consultations, the underdiagnosis is even more pronounced.

In Québec, there has been no systematic effort to identify affected families. Determining the exact number of affected individuals is all the more difficult because there is no register or parents' association that could provide such data. Furthermore, the medical management, but also the social and educational management, of affected individuals seems extremely decentralized, and there is no specialized centre to which all of these people are referred (see Sections 5.3, 5.4 and 5.5 and Appendices IV and V). Based on informal discussions and data gathered by means of a questionnaire sent to a number of health professionals, the specialists concerned (geneticists, neurologists, pediatricians, etc.) have, with a few exceptions, few patients with this diagnosis in their clientele[83]. The discussions and questionnaire did not therefore enable us to reliably estimate the number of Quebecers diagnosed with the syndrome. We shall see below the extent to which the data gathered from laboratories can assist us in this regard.

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[82] These data are compiled by the Régie d'assurance-maladie du Québec (RAMQ) for administrative, not scientific, purposes. The RAMQ issues several warnings regarding the use of its statistics. First, physicians do not systematically provide these data, especially when a number of problems are discussed during a given consultation. Second, these data reflect the number of visits outside hospitals, not the number of patients concerned. Lastly it could be that, for developmental delay, codes other than 315.9 are used on occasion, since this code concerns non-specific developmental delay (which excludes, for example, delays attributable to neurological causes). In short, these data therefore probably reflect only a portion of the services devoted to this patient population.

While the number of affected individuals is uncertain, Québec does have a reliable estimate of the premutation prevalence. A study carried out in the Québec City area involving more than 10,000 samples determined the premutation prevalence to be 1 per 259 females (95% confidence interval: 1/373 - 1/198) (Rousseau et al., 1995).

In short, the exact number of Quebecers with fragile X syndrome is difficult to determine, given that there is no register, association or specialized centre that could provide such data. According to a theoretical calculation based on the latest prevalence estimates, there were, in Québec, in 2000, 250 affected children under the age of 15 years, for a total of at least 1,377 affected individuals.

5.2 ORGANIZATION OF LABORATORY SERVICES

Prior to 1991, fragile X syndrome was diagnosed by cytogenetic techniques (see Appendix II) performed in pathology or sometimes hematology laboratories. When molecular diagnosis became available[84], clinicians first used a laboratory outside Québec. However, in late 1991, molecular diagnostic services became available at the Biochemistry Unit[85] at the Saint-François d'Assise Branch of the Centre hospitalier universitaire de Québec (CHUQ). Also, since 1997, a fragile X test has been available at Hôpital Sainte-Justine's molecular biology laboratory[86]. Lastly, the private company Procréa Biosciences Inc. offers this molecular test, but it has it done by other laboratories.

5.2.1 Overview of the operations of the laboratory of the Biochemistry Unit, Saint-François d'Assise Branch, Centre hospitalier universitaire de Québec (CHUQ)

Since 1991, this laboratory has been using the reference method discussed above (see Section 3.4, Chapter 4, and Appendix II)—the Southern blot protocol described by Rousseau (Rousseau et al., 1991). It is used on all samples and is followed, if need be, by PCR with polyacrylamide gel electrophoresis in order to accurately determine the size of normal alleles and small expansions.

From 1991 to August 1999, 3,825 molecular analyses of the FMR1 gene were performed. The laboratory responds to requests for molecular tests from all parts of Québec. The annual number of requests gradually increased from 256 in 1992 to 500 to 600 since 1996.

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[83] The vast majority of specialists apparently follow only a few patients and perhaps their families. There are rarely more than about 20 of them in a clinic's clientele, even at university hospitals. One exception is the Neurogenetics Unit at the Centre hospitalier universitaire de Sherbrooke, for a greater effort has been made to identify this population in the Eastern Townships. In August 2000, the clinic's patient population included 69 affected adults and 43 affected children, including 19 adults and 21 children with a full mutation, 49 adults and 20 children with a premutation and, one adult and two children with a mosaic. It is estimated that this number of people involves about a dozen founder families, but it is difficult to accurately determine the number of families because some individuals may be more-distant relatives.

[84] Before direct molecular tests based on the identification of the dynamic mutation were developed, cytogenetic tests were briefly supplemented by linkage analysis, which is based on identifying markers near the gene. We will not, however, discuss this approach any further in this report.

[85] Originally, the Molecular and Human Genetics Research Unit.

[86] Originally, the pathology laboratory.

Based on the data gathered by the laboratory, 11.6% of the tests are performed for people with a family history of the syndrome. Up until now, 4.4% of the tests have been considered urgent. They include fetal specimens and specimens from pregnant women with a family history of mental retardation or a family history of the syndrome.

In all, 38 prenatal diagnoses have been performed. Of these, 22 fetuses were found to have normal alleles, eight had a full mutation, including one with a mosaic, and four had a premutation. In three cases, the results of a chorionic biopsy had to be confirmed by amniocentesis, twice to determine the methylation status and once because of maternal contamination. Also, in four cases, a reliable diagnoses could not be made following amniocentesis because of an insufficient quantity of cells in two cases and suspicious but atypical results for two other specimens[87].

Thirteen to 19 new families continue to be identified each year, with the result that a total of 117 families are known to the laboratory.

The results of the tests performed for clinical purposes since the laboratory began operations are presented in Table 5.2. Although the number of tests performed in males is nearly two and a half times greater than the number for females, the proportion of tests revealing a full mutation or a mosaic is as large, if not larger, in females (5.3% vs. 4.3%). However, the reasons tests are ordered might differ to a certain extent, which seems to be corroborated by the age distribution of the people in whom a full mutation or a mosaic has been identified (*Table 5.3*). In males, 72% of the diagnoses are made before the age of 15 years, which suggests that most of the tests are ordered for symptomatic individuals, whereas, in females, the diagnoses tends to be made later. The females (57%) diagnosed between the ages of 15 and 65 no doubt include more asymptomatic or unsymptomatic persons identified in the context of family screening.

However, it seems, based on the information obtained by the questionnaire completed by some of the professionals concerned, that, in the past few years, fragile X syndrome has been diagnosed more and more in preschool-age children and that greater attention is being paid to evaluating girls.

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[87] In all, for 38 female patients, 18 chorionic biopsies were performed as well as 23 amniocenteses, three of which to confirm chorionic biopsy results. Among these amniocenteses, one analysis had to be repeated, on extra cell culture, and four did not permit a conclusive diagnosis.

Table 5.2: Number of tests performed from 1991 to August 1999 and the results by gender

	FMs	Mos.	PMs	Deletion	Normal	Total
Females	51	7	140	1	894	**1,093**
Males	91	25	33	1	2,580	**2,730**
Total	142	32	173	2	3,474	**3,823**

Legend: **FMs**: full mutations; **Mos.**: mosaics; **PMs**: premutations.
Source: Biochemistry Unit, Saint-François d'Assise Branch,
Centre hospitalier universitaire de Québec (CHUQ), November 1999.

Table 5.3: Number of individuals, by gender and age group, in whom a full mutation or a mosaic was detected

Age group	Males	Females	Total
0-5 years	50	9	59
6-14 years	32	14	46
15-65 years	31	31	62
66 years+	0	1	1
Total	113	54	168

Source: Biochemistry Unit, Saint-François d'Assise Site,
Centre hospitalier universitaire de Québec (CHUQ), November 1999.

5.2.2 Overview of the operations of Hôpital Sainte-Justine's molecular biology laboratory

In 1997, this laboratory instituted a test using the PCR method first, with confirmation by the Southern method when a premutation is detected or when a full mutation is suspected. This method is inspired by a technical note by Hilbert and Sabine (1996) and is based on amplification with a DNA-polymerase that is more stable at high temperatures, this to improve amplification of the trinucleotide expansion, and on visualization of the amplification products, which are labelled by incorporating radioactive nucleotides. It is, therefore, an alternative method, whose validity has not yet been documented in the literature. The laboratory heeds the usual precautions concerning PCR and undertook, in 1999, a project to validate its method, using a relatively small number of samples, in collaboration with the CHUQ laboratory. The preliminary results of this study indicate that, for a large proportion (approximately 25%) of samples containing allelic mosaics, based on the reference method, the alternative method has revealed only premutations but not full mutation alleles (Personal communication, Dr. F. Rousseau, Saint-François d'Assise Branch, Centre hospitalier universitaire de Québec, and Dr. L. Oligny, Hôpital Sainte-Justine, Montréal). The impact that these classification errors can have on the prognosis justifies the decision on the part of the laboratory's management to review their diagnostic protocol and systematically check previous samples that tested positive for premutations. The protocol will therefore be modified, taking the advantages of the reference method into account.

By the end of November 1999, the laboratory had performed a total of 687 molecular analyses of the FMR1 gene, the number of requests having increased from 178 in 1997 to 260 for the first 11 months of 1999. In the beginning, the tests the laboratory performed were requested mainly by physicians at the hospital, but the number of requests from other Montreal hospitals and from clinics in that city and its surrounding area currently account for about 10 to 20% of all the requests. Eight females and 26 males (or 4.95% of the individuals tested) have been diagnosed with a full mutation, and 13 females and 4 males have been found to have a premutation. The number of corresponding families and the proportion of tests ordered because of a family history of the syndrome are unknown. A single prenatal diagnosis has been performed, on an amniocentesis specimen.

5.2.3 Test demand forecast

The number of tests ordered annually has increased considerably, reaching 700 to 800 since the past few years. This increase is no doubt due to increased knowledge of the syndrome by health professionals, to the dissemination of information about the molecular tests and to recommendations issued by professional associations in the United States concerning the indications for these tests. In this regard, there is apparently, at the present time, an increasing number of requests for diagnosis in children with developmental delay and learning disabilities, and even autistic tendencies or attention deficit with or without hyperactivity. This trend is reportedly observed at several university hospitals, and test requests do not come just from geneticists and the various pediatric specialists, but also from first-line pediatricians, child psychiatrists, obstetricians and general practitioners.

Upon comparing the anticipated number of affected individuals in Québec (Table 5.1) with the approximate number of individuals in whom a diagnosis has been made based on laboratory results, it is seen that the syndrome is underdiagnosed in Québec, as it is elsewhere. Given that some families have not been identified and given the trend, observed at most specialized centres, toward earlier diagnosis based on broader indications, the demand for tests is likely to remain stable or continue to grow in the next few years. In addition, if information continues to be disseminated, this could translate into more-systematic molecular testing in diagnostic work-ups for mental retardation and developmental delay and into a more rigorous application of the recommendations relating thereto. The current situation could also change due to an increased knowledge of the syndrome on the part of professionals in schools and day-care centres, with psychologists and speech therapists, for example, referring children with problems or delays more often.

Other factors, such as access to tests and the adoption of new diagnostic or screening strategies, could affect the demand for analyses of the FMR1 gene. Access to a test depends, among other things, on a laboratories' ability to meet the demand for them and the conditions for financing them. The cost of molecular testing is substantially lower than that of cytogenetic analysis, even if the reference method used is relatively labour-intensive and requires more time than certain alternative methods.

The entire operating budget of Hôpital Sainte-Justine's molecular biology laboratory comes from the hospital's budget, but some of the equipment predates the merger of various molecular laboratories and had been paid for, at the time, with research funds. A $250 charge is billed to hospitals that request karyotyping and molecular analysis of the FMR1 gene. The laboratory at the Saint-François d'Assise Branch of the Centre hospitalier universitaire de Québec (CHUQ) originally provided the service for fragile X syndrome by operating on research budgets and using an infrastructure financed by research funds. Currently, the operating expenses are absorbed, in part, by the Biochemistry Unit, with the rest covered by interhospital billing. In fact, most of the test requests come from outside CHUQ, and there is a charge of about $100 for testing an index case. However, the tests on relatives are performed free of charge. These costs are defrayed by the requesting hospitals with funds generally from a closed budget reserved for genetic diseases or from the medical biology budget. One factor that could limit the demand for tests, but especially compromise equal access to them from a geographical standpoint, is the allotment of these specific budgets reserved for genetic diseases and the ceiling put on them.

The above-mentioned laboratories are able to continue meeting the current demand for tests, but a marked increase would require recruiting and training additional staff. However, it would not be desirable to increase the number of laboratories offering the tests, given the expertise required to perform them and interpret their results and the importance of close collaboration between research laboratories and clinical laboratories. However, test demand could change substantially should new diagnostic or screening strategies be adopted, e.g., the screening of pregnant women. This possibility is discussed in Chapter 6.

Recap

The data gathered from the laboratories that perform molecular tests in Québec provide an approximate estimate of the number of individuals diagnosed with fragile X syndrome (a total of 142 males and 66 females). Naturally, these data are not exhaustive, since a certain number of diagnoses have been made on the basis of cytogenetic tests[88] or at laboratories outside Québec. However, a comparison of these data with the theoretical estimates based on the syndrome's prevalence (910 males and 467 females) does seem to confirm that the syndrome is underdiagnosed in Québec. Also, considering the number of individuals in whom these tests have been performed (4,510 in all) and the number of individuals who might be mentally retarded or developmentally delayed, it is clear that only a fraction of the at-risk population has been tested.

Two laboratories offer molecular tests for the FMR1 gene: CHUQ's Biochemistry Unit laboratory (Saint-François d'Assise Branch) has been offering a test since late 1991, and Hôpital Sainte-Justine's molecular biology laboratory developed a test in 1997. The reference method is currently used at the Saint-François d'Assise Branch, whereas at Hôpital Sainte-Justine's molecular biology laboratory, an alternative method in which PCR is performed first and whose analytical validity has yet to be documented in the literature was used initially. However, in light of the preliminary results of a recent validation project carried out jointly with the CHUQ laboratory, the management of Hôpital Sainte-Justine's laboratory plans to modify the diagnostic protocol.

The number of tests requested annually has gradually increased, reaching 700 to 800 since the past few years. It seems that an increasing number of tests are being ordered because of developmental delay and learning disabilities and also that there is a trend toward earlier diagnosis. The demand will probably remain stable or continue to grow in the next few years. The funds allocated for interhospital billing for genetic tests are one of the factors that could limit the demand for tests and compromise equal access to them. Lastly, the laboratories can presently meet the demand, but should it increase substantially, additional personnel would have to be hired and trained.

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[88] Many authors agree that the results of cytogenetic tests should be confirmed by molecular analysis, but some of them use molecular analysis only in cases of ambiguous results or a discrepancy between the results and the clinical findings. Also, these checks are not necessarily easy from a logistical standpoint. A number of laboratories used to perform these cytogenetic analyses in Québec, and even if we knew the number of diagnoses made by cytogenetic analysis, we would still not know how many of them were confirmed or ruled out by molecular analysis.

Given the anticipated increase in test demand based on both the current underdiagnosis of the syndrome and the ordering recommendations issued by professional associations, it may be justified to support two laboratories performing these tests in Québec. However, it would be desirable for the diagnostic protocols to be harmonized and for a quality control mechanism to be put in place.

5.3 ORGANIZATION OF CLINICAL SERVICES

For families with a child who has problems consistent with a diagnosis of fragile X syndrome, clinical services are involved at three different stages: the diagnostic workup, family evaluation and counselling, and the clinical follow-up of the affected individual once a diagnosis has been made. As for access to clinical services for the diagnostic workup, it should be noted that there is no referral centre dedicated to the syndrome and that numerous points of entry into the healthcare system can be used by families with a child with mental retardation or other suggestive signs. In addition, there is a great deal of diversity in the recruitment channels (contacts and referrals made in medical, school and preschool settings) leading to an assessment of the problem and to a request for a test to confirm or rule out fragile X syndrome.

This diversity is due, among other things, to the fact that this syndrome exhibits a very broad spectrum of manifestations ranging from a simple learning delay to the classical clinical manifestations of the syndrome. When the first symptoms appear, the parents will usually consult their family physician or a pediatrician. They may then be referred to specialized—neuropediatric or pedopsychiatric—services or to development clinics. How exhaustive the diagnostic workup proposed for the speech delay, developmental delay or mental retardation will depend on the type of professional consulted. As mentioned in Section 3.3, efforts have been made over the past few years to promote a consensus regarding the optimal diagnostic workup for developmentally delayed or mentally retarded children (Curry et al., 1997), but it will no doubt be some time before these efforts result in the standardization of practices in the field. Besides, it is not impossible that such a workup will have to be seen more as a stepwise process, with referrals to various superspecialties, depending on the signs associated with the developmental delay or mental retardation (e.g., neurological, behavioural or dysmorphic signs). As for the complete assessment of the child's cognitive and adaptive skills, it can be done at specialized clinics in tertiary hospitals, but some parents consult private psychologists instead, since the waiting lists for hospital-based psychologists are very long.

Parents with a developmentally delayed child often find the period leading up to the clinical diagnosis and the potential confirmation of mental retardation extremely painful because of the anxiety due to the diagnostic and prognostic uncertainty, the seriousness of the issues for their child's and the family's future, the bouncing from one medical specialty to another, and the time it takes to reach a definitive conclusion. Yet, from a medical standpoint, the diagnostic workup is often done very gradually, except in the more severe cases, because of the insidious onset of the first symptoms and significant overlapping between the normal rate of development and the precursor signs of mental retardation, i.e., developmental or speech delay. There is also a desire not to needlessly alarm the family and hastily label children who develop more slowly than average but who eventually catch up with their peers. Nonetheless, a more systematic approach to the workup would, perhaps, lead to a shorter waiting period and less bouncing from one specialty to another while promoting a more efficient use of the resources involved.

When a family history of mental retardation or fragile X syndrome is known or when a diagnosis of fragile X syndrome has been made, the family is usually referred to a genetic service. Depending on the case, medical genetic services can therefore be involved in the diagnostic workup, but they are called upon more to provide genetic counselling and to screen for carriers in the family. Insofar as a limited number of affected families are referred to geneticists, the latter are generally able to meet the current demand, but since they are few in number, they could probably not be involved more actively in the diagnostic workup of all children withmental retardation or developmental delay. Also, in the fragile X families, not all the individuals concerned have availed themselves of genetic counselling. This can be explained by a set of factors, most of which have to do with the relatives' request for counselling, which depends considerably on the quality of communication between the family members for sharing information and on the need relatives feel to consult. These needs arise especially when a woman who is pregnant or wishes to become pregnant wants to know if she has a premutation.

The medical management that follows a diagnosis consists of a regular follow-up for monitoring the syndrome's usual clinical manifestations and for preventing complications (AAP, 1996; Hagerman, 1996a; de Vries et al., 1998b). In fragile X syndrome, it is the behavioural problems which, after early childhood, usually prompt the medical follow-up. Depending on the predominant problems, the follow-up can be done by a geneticist, a pediatrician, the family physician, development clinics or other specialists involved in the diagnostic process. A recent survey of parents of mentally retarded children indicates that they often deplore the manner in which the diagnosis is communicated and the lack of psychosocial support provided on this occasion, and the lack of referrals to other sectors that might meet their needs (Bouchard and Pelchat, 1994; Perreault, 1997). The physicians involved are not always surrounded by a team of professionals who can participate in the psychosocial support, and even if they are, timely access to these services is often compromised by long waiting lists. As for the follow-up, the services are sometimes coordinated by a coordinating nurse (at specialized clinics) or by the attending physician, but there is generally little contact between the medical sector and the other partners in day-care centres, schools, local community service centres (CLSCs) and rehabilitation centres.

Recap

With regard to access to diagnostic services, there is wide diversity in the recruitment channels and points of entry, which can be explained mainly by the syndrome's broad spectrum of manifestations and the fact that its expression varies according to the child's stage of development, but also by the lack of centralized services[89]. Furthermore, the diagnostic workup depends, to a certain extent, on the physicians consulted, and we are led to believe that standardizing practices and/or disseminating more widely protocols for evaluating developmentally delayed or mentally retarded children could speed up the process and promote more efficient resource utilization and reconcile the children's best interest and the parents' needs.

As the services are presently organized, geneticists are meeting the needs expressed by affected families, but a complete evaluation of these families is not always done. Follow-up services are more uncertain, as they depend on the professionals initially consulted, and psychosocial support is sometimes difficult to obtain in a timely manner. Lastly, the lack of communication and collaboration between the medical sector and the reeducation/rehabilitation sector often results in a lack of referrals to reeducation and social integration services after a diagnosis is made.

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[89] These access to diagnostic services problems have been reported elsewhere and are therefore not unique to Québec (Bailey et al., 2000).

5.4 ORGANIZATION OF REEDUCATION[90] AND SOCIAL INTEGRATION SERVICES

As explained in Section 1.5, some of the services required by fragile X individuals are beyond the scope of medical services as such, involving, for example, functional reeducation and social integration services and psychosocial support for the patient and his or her family. Intervention by a number of professionals may therefore be necessary, depending on the affected individual's age and clinical presentation and the support the family receives in the community.

Different sectors of the health and social services system provide reeducation and social integration services, mainly rehabilitation centres, but also hospitals and CLSCs. Some of these services can also be obtained through the school system, which will be discussed in Section 5.5. In Appendix IV, we review the role of rehabilitation centres and the involvement of the other sectors in this regard. We also discuss the policies and goals underlying the changes that have occurred in the past few decades in services for the mentally impaired and which have been marked especially by a desire to deinstitutionalize them in order to promote their social integration. Below, we shall focus on the impact of the current organization of services on families in the grips of fragile X syndrome.

Under *An Act respecting health services and social services*, the mission of rehabilitation centres is to offer adjustment, rehabilitation and social integration services to persons who, by reason of an impairment, require such services, as well as persons to accompany them, or support services for their families and friends[91]. To this end, they are to assess these individuals' needs and ensure that the services are offered, either at the rehabilitation centre, in the individual's community or by other persons or organizations capable of assisting them. If several resources are involved in providing services, the institution which dispenses most of them, or a professional designated jointly by the resources concerned, must develop an individualized service plan (ISP)[92], with each institution developing its own intervention plan (IP).

Since fragile X syndrome can first manifest as speech delay and developmental delay and since mental retardation is often documented only when the child has reached school age, two types of rehabilitation centres may be involved with these patients: rehabilitation centres for the mentally impaired and rehabilitation centres for the physically impaired. It is on the basis of the impairment, mental or physical, that services are provided. Knowledge of the specific etiology is not a determining factor in access to services.

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[90] We have adopted here a terminology that is not widely used in Québec. We wanted to be able to easily distinguish between two types of services whose preferred time and type of intervention, on the one hand, and whose distribution and accessibility, on the other, are not comparable. The term *reeducation services* will refer here to services offered by speech therapists, occupational therapists and physiotherapists. While the ultimate goal of all the services is the social integration of people with a handicap, reeducation services basically concern their impairment and disability, while social integration services concern more (not exclusively) their handicap. Furthermore, for fragile X individuals, reeducation intervention is crucial in early childhood and throughout childhood, while social integration services will be useful throughout their lives. Lastly, reeducation services are available, as discussed below, in the medical and school sectors and at rehabilitation centres (for physical more than mental impairments), but they are relatively inaccessible. As for social integration services, they are mainly offered by rehabilitation centres and CLSCs, except for work integration, which requires the involvement of several partners.

[91] R.S.Q., c. S-4.2, s. 84 (Québec).

[92] R.S.Q., c. S-4.2, s. 103 (Québec).

In reality, services dispensed directly by rehabilitation centres for the mentally impaired (RCMIs) consist mainly of family support[93] and early stimulation for younger children[94], and, starting in adolescence, vocational counselling[95] and residential support (FQCRPDI, 1994). Rehabilitation centres for the physically impaired (RCPIs) provide, among others, services to people with speech or language impairments or multiple impairments. The services provided are for both functional reeducation (speech therapy, occupational therapy, etc.) and social integration/rehabilitation.

As for developmentally delayed children, the situation is not as clear. The Fédération québécoise des Centres de réadaptation pour les personnes présentant une déficience intellectuelle (FQCRPDI) and the Fédération de la réadaptation en déficience physique du Québec (FRDPQ) published, in 1996, common goals stating that it is the responsibility of rehabilitation centres for the physically impaired to follow developmentally delayed children as long as serious indicators of mental retardation have not been detected (FRDPQ and FQCRPDI, 1996). This agreement has been implemented in only a few regions.

It is also possible to obtain reeducation and psychological services in hospitals, but access to them is generally hampered by long waiting lists. The CLSCs are responsible, in the area of home care and family support, for providing child respite care and psychosocial support, which are especially important for families in the grips of fragile X syndrome. CLSC social workers also play a substantial role in terms of referrals to the most appropriate services and guidance for obtaining financial support, two areas where the provision of information must be accompanied by concrete assistance with the administrative legwork. Considerable differences, which are indicative of the CLSCs' preferential aims with regard to this overall mission, nonetheless exist in the services available to mentally retarded individuals.

Thus, as with clinical services, there are, in some regions, many points of services and a host of possible paths to reeducation and social integration services. Coordinating these services is a huge challenge. Although RCMIs often do this coordination for the users they serve and although their mission requires that they work in tandem with other organizations, collaboration with these organizations is not always well established, despite measures taken at the structural level and at the individual-management level.

At the organizational level, cooperation efforts were made in the late 1980s by way of the creation of access mechanisms, the development of regional service organization programs (RSOPs) and the agreement between the FQCRPDI and the FRDPQ concerning the management of developmentally delayed children (FQCRPDI and FRDPQ, 1996)[96]. However, in practice, these efforts resulted in solutions which are highly region-dependent, which have not been systematically implemented in the field, and which have not received the necessary follow-up (Lamarre, 1998). On the whole, good collaboration is beginning to take hold between the RCMIs and CLSCs[97], but collaboration between rehabilitation centres, specialized clinics and schools still seems very fragmentary and nonfunctional. For example, several special observers agree that, because of budget cuts and to prevent service redundancy, the RCMIs have a tendency to leave the responsibility for children reaching school age to the schools[98]. The lack of collaboration during this transition results in a break in the continuity of reeducation services, disrupts the service plan that may have been developed and forces parents to go through the process of obtaining new services (Perreault, 1997).

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[93] Family support and especially respite care are also the responsibility of CLSCs.

[94] However, RCMIs rarely have a speech therapist on staff, with the result that the services provided during early childhood may, perhaps, not meet the most urgent needs of fragile X children, who generally have a serious language acquisition delay.

[95] Other organizations also play a role in work integration programs (the Ministère de l'Éducation, the Ministère de la Sécurité sociale, Emploi Québec and the Office des personnes handicapées du Québec).

[96] It is reported that a few regional consultation meetings have also involved community organizations, municipalities, youth centres and mental health services (Lamarre, 1998). Other players that could be involved at the regional

As regards the management of the individuals concerned, the individualized service plan (ISP) is *a priori* an excellent tool, not only for adapting services to the individual's needs, but also for coordinating these services, since, in principle, all the resources involved take part in developing the ISP and since the plan constitutes, in a way, a commitment to provide services. However, a service plan is developed for only a minority of the persons concerned. Their involvement in the process or their family's involvement still varies, and this tool alone cannot, of course, solve the problem of accessibility to services for which resources are not available (Lamarre, 1998). Lastly, the efforts made, both by organizations and professionals, generally concern only the health and social services system. And intersectorial coordination is less than satisfactory[99].

As for the available services, the surveys conducted at the MSSS's request (Perreault, 1997; Lamarre, 1998) showed that support for the mentally impaired and their families is one of the weak links in the current system. Because of the budget cuts over the past few years, both in the health and social services system and the educational system, waiting lists are very long due to a lack of human and financial resources. Speech therapy, in particular, and occupational therapy and psychological services are particularly difficult to obtain both at hospitals and at RCPIs (Perreault, 1997; Lamarre, 1998). Furthermore, because of the low availability of these professionals and in line with a competence-based approach that encourages the parents' full participation, the latter must often assume part of the reeducation care, the professionals teaching them the exercises and thus limiting their interaction with the child to a periodic assessment of his or her needs, with the services adjusted accordingly. Many parents complain that they are thus deprived of the opportunity for respite, yet respite care services and social support are some of the most difficult services for these families to obtain. As regards RCMIs, the adult clientele, which is in the majority, seems to be affected more by long waiting lists than children, although problems are also reported for early stimulation services and housing resources.

In addition to the waiting lists for specialized services, the lack of human and financial re-

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level in these consultations are child care services, school boards, the job sector and the leisure-time activities sector.

[97] Following the institution of specific access mechanisms to the RCMIs, the CLSCs became the preferred points of entry for access to the services dispensed or coordinated by RCMIs.

[98] For a certain number of children, rehabilitation centres continue to offer home education assistance services.

[99] However, a dialogue is underway between the Ministère de l'Éducation (MEQ), the Ministère de la Santé et des Services sociaux (MSSS) and the Ministère de la Famille et de l'Enfance for the purpose of reviewing the MSSS's social integration policy and the MEQ's school adaptation policy. In the MEQ's new school adaptation action plan (MEQ, 1999a), a certain number of areas are identified that will be the subject of more-thorough consultations, such as services for handicapped 4-year-olds and support for personnel working with children with attention deficit.

sources is also leading to a high level of staff turnover, which hinders service continuity (Perreault, 1997). Service continuity is important for children with developmental problems, and we know that fragile X syndrome children are particularly sensitive to the stability of their environment, which stability is already compromised by the normal transitions that come with age (day-care centre → school, school → workplace, etc.), but also by the frequent re-referrals between various points of service. Lastly, budget cuts, together with the coordination problems mentioned earlier, result in organizations offloading their responsibilities and services vis-à-vis certain populations (Lamarre, 1998). The populations most vulnerable in this regard are those that are not perfectly in line with the missions of the existing organizations, either because they have rare, complex or poorly understood problems or because they have multiple impairments. These populations, which include fragile X families, are therefore at risk for falling between the cracks and not being able to avail themselves of the resources they need. While access to services seems less of a problem for certain syndromes that are better characterized, such as Down's syndrome, all of the problems mentioned above have been identified as part of an action plan specific to autistics (Morin, 1996). Autism[100] and fragile X syndrome are examples of diseases that affect development which are difficult to diagnose, which are variable in their presentation and whose management can involve professionals in a number of sectors.

Recap

Thus, several types of organizations offer reeducation and social integration services. It is very likely that the services which fragile X families use vary enormously. Naturally, their needs are not identical, but the services received seem to depend mainly on the professionals contacted when the problem is identified (point of entry) and the organizations they are subsequently referred to.

Despite the numerous points of service and the coordination efforts in the health and social services system, parents of fragile X children face many problems. First, given the nature of the syndrome, they are especially prone to offloading and exclusion because of the rehabilitation centres' definition of target populations. Second, like other parents of handicapped children, they have to bear the consequences of a lack of resources for certain reeducation (waiting lists for speech therapists, psychologists, occupational therapists) and social integration services. Lastly, they unfortunately often find themselves alone, without help, direction or adequate information, when it comes time to do a good deal of administrative work in order to obtain services or ensure their continuity. As regards social services, the weak link is, for that matter, support for the families, who already have to look after their children on a daily basis.

Efforts to improve service coordination and continuity are underway and should be encouraged. In the case of fragile X syndrome, better knowledge of the heterogenous and progressive nature of the syndrome could bring out the specific needs of this population and the necessity to rethink responsibility sharing with respect thereto. If this were to happen, we could expect that more-systematic diagnosis through molecular testing could improve access to reeducation and social integration services and their planning.

5.5 ORGANIZATION OF EDUCATIONAL SERVICES

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[100] Autism is characterized especially by developmental delay in social interactions, communication problems (including language acquisition delay) and a limited, repetitive and stereotyped activity, interest and behaviour repertoire. It is accompanied by mental retardation in 75% of cases (Morin, 1996). Unlike fragile X syndrome, autism is a combination of behavioural manifestations with no known etiology or biological markers, which makes diagnosing and defining the syndrome even more difficult (Feinstein and Reiss, 1998).

In order to determine the type of educational path taken by fragile X children and the nature of the services they receive during their education, we will have to rely, to a large extent, on data concerning the education of handicapped students and students with social maladjustments or learning disabilities (HSSSMLD). Statistics from the Ministère de l'Éducation du Québec (MEQ) are available according to this classification, not according to the etiologic diagnosis. From the data available for the places of education of HSSSMLD, which are presented in Appendix V, we shall attempt, below, to extrapolate the situation experienced by fragile X children and to analyze the problems that their families face.

In Appendix V, we also briefly review the legislation governing educational services and the ideas and policies that have guided the changes that have occurred over the past few decades. We have witnessed the transition from a segregated school system to integration of handicapped children in regular schools and classes, but this transition has not occurred at the same pace in all communities and for all types of handicaps. Furthermore, there persist clashes between different lines of thought, with the result that the debate concerning school integration is not over and that changes can still be expected.

The Ministry's goals and policies support school integration based on as normal an educational path as possible and a double (MEQ, 1978) or even triple[101] educational objective, which should foster both learning and social integration. The school boards are legally required to adapt the educational services to the needs of handicapped students and those with learning disabilities, but they have a certain amount of leeway in choosing the services to be dispensed, as two different modalities are provided for in the law: integrating students into regular classes, with various forms of student or teacher support (integrated services), and grouping students together in special classes or schools (segregated services). Although integration was opted for in the *Education Act*[102] updated in 1999 and in the 1999 policy on special education (MEQ, 1999b), all the measures set out in the action plan for special education will be implemented gradually over a 5-year period (MEQ, 1999a). In practice, we can consider that the approaches chosen still differ widely from one school board to another, ranging from the offer of a range of services to the exclusion of one of the two modalities. Regional differences were, in fact, quite significant (Garon, 1997). Once the choice of school is made (the final decision rests with the school boards), its principal has the duty of drawing up the intervention plan suited to the student's needs, with the help of the child, his or her parents and the personnel at the school who will be providing services to the child.

Overall, according to Ouellet (1997), about 1.30% of the public school population at the preschool, elementary and high school levels have a handicap, and about 11% have learning disabilities or social maladjustments. Reportedly, 1.13% of the total public school population have mental impairment identified as such. However, undercounting is very likely, especially in the case of mild mental impairment. More handicapped children are being integrated into regular classes than in the past (see details in Appendix V), although the integration of children with mental impairment and with multiple impairments is proceeding more slowly than for other types of handicaps. However, the type of schooling chosen depends considerably on the educational level (extent of integration clearly lower in high schools than in elementary schools) and on the degree of impairment (integration less frequent if the impairment is more severe or if there are associated behavioural problems).

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[101] In addition to the students' education and socialization, their qualifications are now part of the schools' mission under Section 36 of the *Education Act*, the latest version of which came into effect in October 1999. This addition is in line with the shift toward success (i.e., going from access by the greatest number to success by the greatest number), adopted as part of educational reform.

[102] R.S.Q., c. I-13.3 (Québec).

With the data at our disposal, we are unable to provide an accurate description of the schooling of fragile X children because they can be found in different categories of the Ministère de l'Éducation du Québec's HSSSMLD classification, depending on their age and dominant symptoms (isolated mental impairment ranging from mild to profound, behavioural problems, learning disabilities, multiple impairments) and because the proposed solutions depend largely on the school boards. Lastly, it is not unusual to see mentally retarded children shifted around from one modality to another throughout their school years (Beaupré et al., 1995).

Although there is certainly a great deal of variation in individual paths, based on MEQ statistics and on the places of education for the categories in which fragile X children are most likely to be found, it is likely that: 1) most fragile X children have a mixed path in elementary school, sometimes being in regular classes, sometimes in special classes; 2) the vast majority of boys, whose mental retardation is, on average, more severe, are in a special school at the secondary level; and 3) the severity of the behavioural problems associated with mental retardation is one of the factors that determines these students' paths.

Whatever the educational path, accessibility to student services is essential for children with special educational needs. The specialized personnel working with HSSSMLD include special education teachers (remedial teachers), reeducation and psychosocial professionals, and support staff (special education technicians, handicapped student attendants, etc.). The quality of integration thus depends largely on the resources devoted to it, and the conditions for success do not always seem to be in place in terms of resources, but also in terms of the motivation and training of all the personnel involved (Conseil supérieur de l'éducation, 1996).

The manner in which educational services for HSSSMLD are currently organized may be fraught with consequences for the families. In principle, they are urged to get involved in developing their child's service plan, but this tool is not always used, and when it is, due consideration is not necessarily given to the parents' opinions[103]. Furthermore, despite the existence of an intervention plan, it sometimes happens that the identified needs are not met because of a lack of resources. At a time when resources are scarce or inadequate, parents are often obliged to go through demanding administrative tasks and negotiations, not only with Social Services, but also with schools or school boards, in order to obtain the services they consider satisfactory. For affected children, the bouncing between various institutions and the sometimes very long commute to specialized centres[104] are seen as destabilizing factors both for their education and social integration.

In addition, the evaluation of educational experiments for children with cognitive problems is, according to many authors, one of the weaknesses of the literature[105], and these experiments generally involve heterogenous groups[106]. The impact that these integrated and segregated paths can have on the development of fragile X children is also not documented. An evaluation in line with the objectives of integration should cover the achievement of the developmental potential, the degree of functional autonomy achieved and the quality of social integration. The impact hoped for in the longer term would be work integration. From this standpoint, the paths currently observed at the high school level probably have little effect, since few mentally retarded children take occupational training and since such children rarely meet the requirements for graduating[107].

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[103] Parent participation is encouraged in the 1999 school adaptation policy, at the level of institutional boards and advisory committees on services for HSSSMLD, as well as in the development and evaluation of intervention plans (MEQ, 1999b).

[104] Since the revision of the *Education Act*, school boards are required, under Section 209, to provide services as close as possible to the place of residence when they use the special service agreements between school boards.

[105] The MEQ has, for that matter, agreed to support the development of school adaptation research (MEQ, 1999a).

[106] This approach constitutes a hindrance not only to research on educational interventions, but also to research on pharmacological interventions and social integration (Dykens, 1995; Hagerman, 1999).

Despite the lack of controlled studies of the efficacy of specific interventions in fragile X children, the experience acquired at certain referral centres (e.g., in Colorado) is prompting experts to recommend individualized educational approaches adapted to the syndrome's characteristics (Wilson et al., 1994; Bailey and Nelson, 1995; Scharfenaker et al., 1996; Abbeduto and Hagerman, 1997). Thus, a certain number of characteristics of these children's cognitive and behavioural development (see Sections 1.3 and 1.5) should be taken into account when developing individualized service plans and when making educational path decisions. For example, let us remember the importance of language problems and developmental delay, which require early intervention by reeducation professionals. Because of problems with abstraction and generalization, and of the relative decline in cognitive and adaptive skills starting in adolescence, adjusting the curriculum fairly quickly is desirable to promote the acquisition—through concrete activities that are relevant to daily activities—of basic skills that will determine the child's future autonomy and his or her social integration. Hypersensitivity to auditory stimuli and the worsening of the attention problems that can result from this warrant integration into small groups. Intolerance of environmental changes underscores the need for a stable and reassuring environment and the importance of adequately preparing all transition phases. On the other hand, because of these children's tendency to mimic, integrating them into heterogenous groups with children with behavioural problems or who are significantly delayed may be less beneficial than integrating them into regular classes in terms of social and behavioural models. These particular aspects underscore the fact that an adapted environment is essential for fostering these children's learning and socialization.

In the mental retardation literature, increasing importance is attached to making an etiologic diagnosis (AAMR, 1992; King et al., 1997). While it is useful to base decisions concerning each child on a thorough assessment of his or her adaptive skills, several authors note the lack of standardized instruments for assessing these skills and point out that knowing the etiologic diagnosis can also help one adjust the interventions, especially for clinical entities—like fragile X syndrome—whose behavioural phenotypes and their evolution over time are the most clearly understood (Baumgardner, 1994; Dykens, 1995; King et al., 1997; Flint, 1998). Although social and educational services were traditionally designed on the basis of the degree of impairment and the disabilities associated with it, there is now a trend, in special schools, to take the underlying etiology into account when planning interventions. However, some parents are reluctant to tell school officials what the etiologic diagnosis is, hoping that they can keep their children longer in regular classes.

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[107] The basic aim of the new school adaptation policy is to foster the success of HSSSMLD in terms of education, socialization and qualifications, recognizing that success can manifest differently according to each student's abilities (MEQ, 1999b). In concrete terms, the Ministère de l'Éducation du Québec has included, in its school adaptation action plan, provisions to facilitate access to vocational training and to secure collaboration with the Ministère de la Santé et des Services sociaux and the Ministère de l'Emploi et de la Solidarité sociale in order to ensure continuity between the schools, rehabiliation centres and the job market (MEQ, 1999a).

Recap

In the current school system, educational services are adapted to the specific needs of handicapped children according to two modalities: integrated services and segregated services. Until quite recently, the school boards had considerable leeway in choosing the modality. The revised version of the *Education Act* and the new policy on special education place greater emphasis on integration into regular classes, but such integration is conditional on the existence of benefits for the student and on the absence of major constraints on his or her environment.

In fact, the practice of integrating HSSSMLD into regular school settings is already much more frequent than in the past, even if it is proceeding more slowly for mentally retarded children. However, the individual paths vary enormously, depending, in principle, on an assessment of each student's skills and needs, but, in practice, largely on the organizational options chosen by the school boards and on the availability of student services.

This flexibility of the system, which is aimed at adapting the services to the children's specific needs, is, in principle, a plus but can also be a disadvantage for the families, since the lack of personnel for providing student services is an obstacle to achieving this objective. The parents are especially affected by this, since they have to get involved more and negotiate in order to obtain these services.

Although it is difficult to accurately describe the situation for fragile X children, it is safe to assume that the integration attempted at the elementary school level becomes increasingly difficult as the manifestations of mental retardation become more pronounced and as behavioural problems arise. The impact of educational interventions on the development of fragile X children has not been rigorously documented, and it is not clear what type of instruction is best for meeting their needs. Nonetheless, empirical experience seems to suggest that the cognitive and behavioural characteristics specific to the syndrome should be taken into account. From this standpoint, making the diagnosis could contribute to optimizing the environment and planning the learning strategies for these children.

5.6 DISCUSSION

Based on the latest prevalence data and on the fragile X syndrome count for Québec, this disease is underdiagnosed, and in many cases, the known families have only been partially evaluated. To remedy this situation, several diagnostic and screening strategies can be proposed (see Chapter 6). Depending on the objective, there are strategies aimed at identifying premutation carriers and those aimed at identifying affected individuals, with subsequent evaluation of the families. The choice of strategy will depend on the anticipated benefits in relation to the current situation. While strategies for identifying carriers are essentially aimed at genetic counselling, those aimed at better case-finding in children with suggestive signs can, in principle, provide a double benefit to the families, i.e., genetic counselling and improved management of the children. One key factor when choosing the type of strategy is therefore to determine if, apart from the benefits associated with genetic counselling, making an earlier diagnosis confers benefits to the affected individual and his or her family. The potential benefits include information and support for the families, obtaining services and even an impact on the affected child's prognosis. In any case, maximizing the benefits requires timely access to testing.

Furthermore, the responsibilities involved in instituting a diagnostic and screening strategy cannot be seen to be limited to the offer of testing and to medical and genetic management, but must cover more globally the services required by affected individuals and their families. Consequently, we summarize below the weaknesses in the current organization of services in order to determine the usefulness of the diagnosis in obtaining services. We then discuss aspects of the situation in Québec that have an impact on the feasibility of the various diagnostic and screening strategies for the syndrome.

Upon examining the situation in Québec, we observe that there is presently no specific medicosocial or educational management of individuals or families affected by the fragile X syndrome. With the movement to deinstitutionalize the mentally impaired and integrate them into schools and society, this management is provided simultaneously or alternatively by the medical community, CLSCs, schools and rehabilitation centres in conjunction with the family. Because of the involvement of all these sectors with their respective responsibilities, and the progressive and multifaceted nature of fragile X syndrome, there are: 1) numerous points of entry and channels leading to a diagnosis; 2) nonstandardized approaches to the diagnostic workup; 3) different management modalities for reeducation and social integration; and 4) several types of educational paths.

This diversity of individual paths does not, as such, pose a problem as long as service accessibility, continuity and complementarity are ensured. These conditions can be fully achieved only if the necessary services are available and efficiently coordinated. Despite the efforts made in the health and social services system to improve organizational coordination, and despite the options available through the development of individualized service plans for promoting service continuity and complementarity at the individual level, we must recognize that the obstacles to coordination hinder access to services and that optimal use of the resources is undermined because they are scattered. Furthermore, the availability of certain services has been reduced because of budget cuts and the shortage of certain human resources in the health and social services and educational systems. Lastly, all of these problems are exacerbated by a lack of communication and collaboration between these sectors.

These service accessibility, continuity and complementarity problems place an additional burden on affected individuals and their families. Furthermore, there is, in Québec, no parents' association that could provide them information or support[108]. Thus, the parents, who already have to look after special-needs children on a daily basis, are often forced to undertake demanding administrative tasks and negotiate, in order to obtain services, with the various sectors responsible for them. As for affected children, the lack of timely services and the continuity problems, which destabilize their environment, could have a negative impact on their development.

Given the current limits in the provision of services to fragile X families and given the follow-up responsibility of those who develop a diagnostic and screening strategy, the usefulness of early diagnosis would be measured not only in terms of the direct benefits to the families (genetic counselling, etc.), but also in terms of the specific needs of these children being met more adequately (with the resulting indirect benefits for the families). In this regard, it must, among other things, be asked whether knowing the diagnosis facilitates access to services and improves their continuity and complementarity or if the diagnosis is useful for developing appropriate educational approaches.

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[108] There is, however, an association and research foundation in Toronto (Fragile X Research Foundation of Canada), and a centre, in Kingston, which does not, to our knowledge, have any members from Québec but which is intended as a resource for interested parents (Fragile X Resource Centre). There are other associations in the United States, Europe and elsewhere.

It seems that while, in the health and social services system, a diagnosis of fragile X syndrome can be of benefit in accessing services more quickly[109], in schools, such a diagnosis, which is confidential medical information whose disclosure is at the parents' discretion, is, in the eyes of some, a double-edged sword, since it can be both an obstacle to integration and an intervention planning tool. Adjusting the educational approaches in light of the diagnosis is of potential benefit. However, the effectiveness of this requires further research.

Making an accurate diagnosis would very likely not suffice to ensure families service continuity and complementarity. Consideration would have to be given, at least initially, to combining the diagnostic and screening strategy with a substantial effort to provide information[110]. Better knowledge of this clinical entity could no doubt solve, in part, the service coordination and intersectorial collaboration problems, since knowledge of all the manifestations associated with the syndrome could make it possible to identify the child's future needs and therefore plan the necessary involvement of the various organizations. From that point on, an accurate etiologic diagnosis could be of help to parents when negotiating for services. Better information should therefore lead, in the medium and long terms, to a better channelling of efforts, better intervention planning and more-rational resource utilization.

Determining the usefulness of the diagnosis in obtaining services for affected individuals, which is discussed above, applies to diagnostic and screening strategies aimed at improving case-finding. Other benefits associated with molecular testing can be achieved as well, thanks to strategies aimed at identifying premutation carriers. All of the strategies will be presented in the next chapter, with a more in-depth discussion of their utility, acceptability and feasibility. Certain aspects of their acceptability will also be examined when discussing the ethical and social issues (Chapter 7). Before detailing the different strategies, we will look again at certain aspects of the situation in Québec that determine their feasibility. Feasibility may be defined as a balance between the resources required for implementing a strategy and the resources that are available or that can be mobilized for this purpose.

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[109] This situation occurs in all the sectors but seems to be due more to the nature of the administrative tasks generally required to obtain specific services than to an orientation adopted by the various systems. Indeed, both in the social and educational sectors, emphasis is placed on the nature of the disability rather than its etiology. Obviously, it is not beneficial for accessibility to these services to be systematically based on the diagnosis, since the vast majority of children in whom the etiology of the mental retardation or developmental delay is unknown would be put at a disadvantage. This does not detract from the potential importance of the diagnosis in planning interventions, in the sharing of responsibilities, in coordinating services and in adapting educational and reeducational approaches.

[110] At least two surveys have shown gaps in health professionals' (Cohen and Loesch, 1999) and special education teachers' (Wilson and Mazzocco, 1993) knowledge of fragile X syndrome. To our knowledge, only one public and professional education program on fragile X syndrome has been instituted, in an American state (Keenan et al., 1992).

Figure 5.1 Schematic representation of the access channels to services according to the mandates of the different organizations involved

This diagram shows that there is no standard service access channel, but rather many points of entry, various referral processes and many interfaces where service coordination takes place. Thus, individual paths vary according to the clinical presentation, the individual's age and the resources initially contacted. The diagram illustrates a few possible steps in the process of obtaining services, depending on whether a speech delay, developmental delay or mental retardation is identified: 1) a visit with a medical specialist for a diagnostic workup and/or 2) a request for reeducation services and psychosocial support at a CLSC (or in the private sector. .); 3) referral to an RCPI or RCMI, depending on the individual's age and the dominant symptoms; 4) referral from the RCPI to the RCMI when serious signs of mental retardation appear; 5) transfer of the file from the RCMI to the school sector when the child reaches school age; 6) responsibilities shared between the RCPI and the school sector in cases where mental retardation has not been established; and 7) the RCMI once again involved, starting in adolescence, for the purpose of providing vocational counselling and preparing the individual for greater residential autonomy.

ABBREVIATIONS:

CLSC: Local community service centre
RCMI: Rehabilitation centre for the mentally impaired
RCPI: Rehabilitation centre for the physically impaired

The data at our disposal regarding the count of affected and at-risk individuals are incomplete, which makes determining the necessary level of resources more complicated. It is therefore difficult to plan the implementation of high-risk-population screening strategies. For example, in schools, special-needs children are counted, but fragile X children may be placed in various categories and the available statistics may be too narrow or too broad. On the one hand, children with mental retardation are, in fact, undercounted, more so during the preschool and elementary years than during high school, which suggests that less serious problems are identified later. On the other hand, more than 10% of students have learning disabilities or adjustment problems, which is probably too high a figure for considering a diagnostic and screening strategy on this basis. More generally, strategies aimed at improving case-finding encounter the problem of selecting high-risk individuals, which is due to the variability in clinical expression and made worse by the regional organization of services for at-risk individuals.

Apart from a lack of data, other organizational aspects of the health-care system complicate the selection process. These include the decentralization of the services involved in assessing mental retardation, the lack of a standard workup of developmental and speech delay, and the absence of a referral centre specific to fragile X syndrome. In the educational system, we also note that there is no specific place, at least for younger people, where a high-risk subpopulation could be defined or targetted. Professionals in this sector do not have sufficient knowledge of the syndrome to participate in the referral process. This problem is specific to strategies that involve diagnosing affected individuals. The issue of selection criteria would not be the same for systematic low-risk-population screening strategies.

As for examining the resources that are available or which can be mobilized for implementing a given strategy, one must check the response capacity and quality of the laboratory services, the diagnostic services and the medical and social follow-up, and that there are effective links between these different services. Up until 1997, there was only one laboratory—which used the reference method—that met the demand for tests for the entire province. The manner in which this laboratory was funded, largely through interhospital billing[111], restricted access to the test and led to the development of other laboratory tests for this syndrome. It was probably as a result of this that another laboratory began to offer molecular testing. Before considering implementing a strategy that requires more diagnostic or carrier tests, one should therefore be sure to have a validated test that is suited to the objective of the proposed strategy and that lends itself to wide-scale use, as well as a sufficient number of qualified personnel for meeting the anticipated demand.

The demand for diagnostic services, genetic counselling and medical and social follow-up could also vary according to the diagnostic and screening strategy chosen. It is fair to assume that a strategy that improves case-finding will require more medical genetics services and perhaps more preschool reeducation services if the diagnosis is made earlier. However, a well-orchestrated strategy might lead to better utilization of the medical resources for diagnostic workup and would therefore not require more such resources. Although such a strategy is not likely to result in an increased demand for social and educational services, since most of these school-age children have already been identified as having special needs due to their cognitive and behavioural problems, certain needs are not being met at this time. Some of the services with limited availability include speech therapy, psychological services and psychosocial support for the families.

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[111] This situation poses a problem if the budget for tests performed in other institutions is limited, as may be the case for certain medical genetics budgets, since the number of genetic tests that can be performed at each hospital is limited.

The neonatal screening network and prenatal follow-up, for which there is a tradition of screening and prevention, constitute already well-established structures and practices that could be used to support other types of strategies, including more systematic approaches.

In summary, the situation in Québec is a determining factor for evaluating certain aspects of the utility and feasibility of the various diagnostic and screening strategies proposed for dealing with the problem of the underdiagnosis of affected individuals and the incomplete screening of families. Given the current deficiencies in the provision of services and given the responsibility of the promoters of a diagnostic and screening strategy, the usefulness of early diagnosis (for case-finding strategies) would hinge on improving the accessibility, continuity and complementarity of the services provided to the families concerned. In this regard, benefits could be achieved as long as a substantial effort to inform professionals enables them to use the diagnosis as a service planning and coordination tool.

As for the feasibility of strategies aimed at improving case-finding, it appears that the lack of available data and some of the current conditions of the provision and organization of services constitute major obstacles to the selection of high-risk individuals. The availability of resources that could be mobilized for testing, diagnostic services and follow-up play a key role in planning and condition the implementation of all the proposable strategies. Of course, each of these strategies warrants a more complete evaluation in light of international experience, using utility, feasibility and acceptability criteria, which will be examined in the next chapter.

6. DIAGNOSTIC AND SCREENING STRATEGIES

The approach usually adopted in medical genetics is to first make a diagnosis in a symptomatic individual referred because of suspicion of a hereditary disease and to then determine the carrier status of the at-risk individuals in this person's family. A distinction can therefore be made between diagnosing affected individuals and family carrier screening. Family screening proceeds systematically, starting with the affected individual (index case), so as to be able to provide genetic counselling to the nuclear family, then to the extended family. This strategy therefore involves both diagnosis and screening, diagnosis in that one seeks to confirm or rule out the presence of a disease in a clinically affected individual or in a fetus, and screening in that it involves identifying individuals who are potential mutation carriers.

Currently, in Québec, the use of molecular tests for fragile X syndrome is part of this familial approach, since molecular analysis has replaced cytogenetic analysis for diagnostic confirmation purposes and since it can also be used to identify asymptomatic premutation carriers, who are at risk for transmitting the disease to their offspring but who may not have been previously identified. The contribution of molecular genetics to genetic counselling is therefore an important one (see Section 3.2). Also, molecular tests are paving the way to other diagnostic and screening strategies, perhaps more systematic, targetting premutation carriers.

In this chapter, we shall describe the various strategies that can be proposed in light of international experience. Although some of them are in widespread use, others were proposed more recently, and their usefulness is being actively debated. The strategies are distinguished by the target population and the method by which it is recruited. The population can be defined on the basis of the level of risk in the target group and/or the stage of life at which it is targetted (prenatal or neonatal, childhood, age of procreation). The method of recruiting the population can be more or less proactive, responding to demand and systematic screening being the two conceivable extremes. The strategy classification used below is based mainly on the objectives. Depending on whether **one diagnoses and screens for affected individuals or screens for carriers at risk for transmitting the syndrome**, the objectives are not the same (see Table 6.1). However, these two approaches are not mutually exclusive, since the strategies for diagnosing affected individuals normally lead to family carrier screening.

As regards **diagnosing and screening for affected individuals**, the impact usually anticipated when making an accurate diagnosis is appropriate medical, psychosocial and educational management of the affected individual. Identifying the source of the problem and giving referrals to the most appropriate resources can alleviate the parents' anxiety. The effect on the affected child's prognosis is an additional benefit, which must be documented for each condition. How early the diagnosis is made depends on the strategy. The advantage of an early diagnosis is the possibility of providing genetic counselling to the nuclear family at the appropriate time. Otherwise, the benefit falls more upon the extended family. These approaches do not prevent all new cases of the syndrome, since they do not permit identification of at-risk families until a first affected child is born. In fact, in the case of fragile X syndrome, a considerable number of identified individuals come from families with no known family history.

Table 6.1: Summary of the types of diagnostic and screening strategies and their objectives

	Strategies for screening and diagnosing affected individuals	**Carrier screening strategies**
Objectives	Early, accurate diagnosis for: ➢ Appropriate medical, psychosocial and educational management ➢ Improving the quality of family life (↘ uncertainty and anxiety; ↗ support and information) [check arrows] ➢ Genetic counselling for the family and cascade screening (if early, benefit for the nuclear family; if not, mainly for the extended family)	Identification of individuals at risk for transmitting the mutation to their offspring for: ➢ Genetic counselling and informed reproductive choices (if prenatal screening and voluntary termination of pregnancy are acceptable: possibility of having healthy children and preventing a recurrence of the syndrome; otherwise, psychological preparation)
Consequences	Benefits limited to families with a history of the syndrome	Secondary impact on prevalence: depends on the choices made by the couples concerned
Types of strategies	1. Current clinical practice of diagnosing 2. Prenatal diagnosis 3. Proactive diagnosis and screening of high-risk populations 4. Neonatal screening	1. Cascade screening 2. Screening of women with a history of mental retardation or other signs 3. Screening of pregnant women 4. Preconceptional screening of women of child-bearing potential

As for carrier screening, its purpose is to identify individuals at risk for transmitting the syndrome to their offspring and, through genetic counselling, to enable them to make informed reproductive choices. At-risk couples of child-bearing potential can have healthy children by availing themselves of prenatal diagnosis, if they so desire. The possibility of preventing a recurrence of the disease in affected families can have the secondary effect of reducing its incidence, but this will always depend on the parents' reproductive freedom of choice.

Sections 6.1 and 6.2 are devoted to describing the different types of strategies for diagnosing and screening for affected individuals and the different carrier screening strategies. For each of these strategies, the scientific, technical and ethical advantages and limitations are discussed and the experience reported in the literature is presented. An overview of the diagnostic and screening strategies is provided in tabular form at the end of each of these two sections (Table 6.2 on page 74 and Table 6.3 on page 83). Section 6.3 discusses the usefulness of the strategies under consideration in light of the aspects discussed above and of the particular considerations for Québec.

6.1 STRATEGIES FOR DIAGNOSING AND SCREENING FOR AFFECTED INDIVIDUALS

Of the strategies used or proposed for diagnostic confirmation purposes, we note the following: **the current clinical practice of diagnosing, proactive diagnosis and screening of high-risk populations, and neonatal screening**. Some of these strategies are strictly diagnostic in nature in that the medical services involved merely act on requests on the part of families, while other approaches are more proactive, involving more systematic recruitment among potentially affected individuals. The current clinical practice of diagnosing is an on-demand strategy, whereas the proactive approach aimed at high-risk populations and neonatal screening involve both diagnosing and screening. However, all of these strategies for identifying affected individuals may lead to genetic counselling of the families and to the offer of cascade screening for potential carriers in the family.

Lastly, **prenatal diagnosis**[112], should be added to this list. It falls within the usual practice of medical genetics and is an extension of cascade screening, since it is normally proposed only after positive carrier status is confirmed in a pregnant woman. We shall start with the strategies commonly used in Québec before discussing proactive high-risk-population screening, with regard to which considerable experience has accumulated at the international level, and neonatal screening, for which the data are more limited.

Several aspects of fragile X syndrome have implications for the choice of strategy for diagnosing and screening for affected individuals. Given that no clinical sign is pathognomonic for fragile X syndrome and given its variable clinical presentation, different indications for performing the test may be considered. The indications for ordering a diagnostic test are covered by guidelines issued by the American College of Medical Genetics (ACMG, 1994) and the American College of Obstetricians and Gynecologists (ACOG, 1996) (see Section 3.3 and Appendix III). Several authors suggest that consideration should be given to broadening the indications to include children with language acquisition delay, learning disabilities or attention disorders. However, relatively few studies are convincingly in support of systematically testing children with these isolated symptoms (see Sections 6.1.1 and 6.1.3). As we shall see, the definition of learning disabilities may also pose a problem. It would therefore be desirable for studies assessing the yield of molecular tests for these broadened indications to continue and for the professional associations to adjust their recommendations in light of the results. In the meantime, in clinical practice, the usefulness of diagnostic testing is being examined on a case-by-case basis in light of the entire clinical picture. Of course, if consideration is given to proactive diagnosis and carrier screening strategies, the usefulness of including these broadened indications would have to be based on a more thorough evaluation.

6.1.1 Current clinical practice of diagnosing

The current clinical practice, which takes place mainly in pediatric settings, consists in responding to a request by parents seeking medical attention because their child is symptomatic. The tention because their child is symptomatic. The request generally results from a referral process that involves medical professionals but also to professionals at a school or preschool.

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[112] We will not discuss here preimplantation diagnosis, since this type of diagnosis has, thus far, been reported rarely for fragile X syndrome (Sermon et al., 1999).

Although some authors have reported their experience regarding fragile X syndrome diagnosis in patients referred to clinics because of mental retardation or developmental delay, this practice has not been systematically evaluated. Often, they merely report the proportion of tests that yielded abnormal results[113], which does not enable one to determine the proportion of affected individuals identified by this method or if the individuals identified are representative of all affected individuals. However, a more exhaustive study, carried out by Cossée et al. (1997), suggests that the syndrome is diagnosed preferentially when there is a familial context and when the symptoms are more suggestive. Consequently, the syndrome is diagnosed more in boys than in girls, and it is diagnosed late[114], with the result that many nuclear families already have more than one affected child. These authors also point out that the selection process leading to diagnostic confirmation varies enormously and that it depends on the referring professionals. While the proportion of test requests due to a family history of mental retardation tends to decrease over time, the proportion of affected individuals in whom a family history of mental retardation is unknown remains stable at about 35% (Cossée et al., 1997). The results of cascade screening following a clinical diagnosis are presented in Section 6.2.1 (Cossée et al., 1997; van Rijn et al., 1997).

The fact that current clinical practice, both on-demand and on-referral, seems to result in late and selective diagnosis suggests that this approach is inadequate for reaching all the people at risk. In 1993, Barnicoat et al. (1993) calculated that only a fourth of developmentally delayed or mentally retarded children[115] had been referred to the regional centre for the purpose of ruling out fragile X syndrome. The weaknesses of this approach are also indirectly confirmed by a few studies in special schools showing a lack of genetic studies in children with learning disabilities (Magnay, 1996). The inadequacy of this approach is further supported by the fact that the diagnosis is often made when there are already two or more mentally retarded individuals in the family[116] (Mornet and Simon-Bouy, 1996).

There are few data on clinical practices that include broader indications, such as behavioural problems, learning disabilities and language acquisition delay. Although there have been several studies involving children referred to specialized clinics, the inclusion criteria used and the analyses performed do not always permit one to draw conclusions applying specifically to these broader indications. For example, Gérard et al.'s study (1997) was based on a cohort of children referred to child psychiatrists but only included children with mental retardation according to DSM-IIIR criteria. Mazzocco et al. (1997), on the other hand, examined children referred to development or pediatric neurology clinics because of learning disabilities. In principle, they excluded all mentally retarded children, based on their verbal and performance IQ scores. However, based on their overall score, about one third of these children were mentally retarded (IQ < 70). In another study, Mazzocco et al. (1998) examined children referred because of language acquisition delay. The total percentage of cognitively delayed children is not mentioned, but the three children identified were mentally retarded or developmentally delayed. Such studies therefore do not provide adequate data for determining the usefulness of performing tests for fragile X syndrome in the absence of cognitive problems on the basis of other indications. They do, however, mention the fact that parents seek medical attention for different reasons, even in the presence of developmental delay, and point out that tests are ordered for different indications.

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[113] The yield (proportion of positive results) varies from 0 to 1.8% in Great Britain (reported by Murray et al., 1997), ~4% in the United States (Kaplan et al., 1994; Brown et al., 1996) and the Netherlands (van den Ouweland et al., 1994),
from 1.9% (Gérard, et al., 1997) to 6.3% in France (Cossée et al., 1997), 8% in Spain (Millan et al., 1999) and even 22% in Italy (Perroni et al., 1996). It probably depends on the age of the individuals tested, the diagnostic indications used and the prescreening done by the referring physicians.

[114] According to the study by Barnicoat et al. (1993) on the reasons for referral and the age at which tests are ordered (cytogenetic at the time), 79% of the test requests were for boys, with 44% of these tests being performed during the preschool years as opposed to only 25% of the requests for girls. In Cossée et al.'s study (1997) of clinical experience from 1991 to 1994, 90% of the requests were for boys, with a mean age at diagnosis of 16 years, which, according to the authors, indicates, in part, a necessary diagnostic catch-up following the introduction of the molecular test.

[115] Based on a conservative prevalence rate of 1% mental retardation or developmental delay.

[116] Of 59 families identified by Mornet and Simon-Bouy (1996), 64% already had two or more mentally retarded members when the diagnosis was made.

Two opposing concerns are expressed in the literature: 1) the desire to make a diagnosis in a larger proportion of affected individuals, including those with less severe signs, and 2) the desire to limit the number of unnecessary tests. Although, in proactive screening projects, use is often made of prescreening checklists, which are based on the family history, a physical examination and/or behavioural criteria, their clinical use is limited[117]. Cossée et al. (1997) do not recommend the use of strict prescreening criteria, trusting the judgment of clinicians, and point out that the test positivity rate should not, as such, constitute a criterion for assessing the value of the referral or selection process, since one must take into account, in terms of benefits, not only the number of affected individuals identified, but also the number of relatives who were able to obtain genetic counselling.

In short, the current practice of confirming a diagnosis of fragile X syndrome by molecular testing is a clinical measure generally in line with American medical association recommendations and meets a need on the part of the families concerned. Although it is a widespread practice, the modalities vary from one referral centre to another and according to the experience of the specialists involved. Few assessments have been reported concerning the consequences of the modalities in this practice. However, the body of experience suggests that the syndrome remains underdiagnosed and that it is often diagnosed late, with the result that identifying a larger proportion of affected individuals early is a major concern. In this regard, broadening the indications for the test is one avenue that has been explored by several authors, but its efficacy is still little documented. This broadening of indications is, to a certain degree, in conflict with the use of prescreening criteria.

6.1.2 Prenatal diagnosis

Prenatal diagnosis (PND) is not a strategy as such, but, like genetic counselling, is part of the current practice of cascade screening of the family following the identification of an affected individual. Prenatal diagnosis can also be part of a more proactive strategy, namely, one for diagnosing and screening for affected individuals or for identifying premutation carriers. Since PND techniques are invasive and involve a certain risk, it is important to first determine the parents' genotypic status and in what circumstances PND is indicated. It is generally recognized that prenatal diagnosis is indicated when a pregnant woman is known to carry a full mutation or a premutation[118]. If the father is a premutation carrier, the usual practice was not to perform prenatal diagnosis, since no expansion into a full mutation had been documented in such circumstances. However, the recent description[119] of a few cases of paternal transmission of a full mutation could drastically change this practice. Further research will be necessary to quantify this risk of transmission and, if possible, to determine the circumstances of such transmission.

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[117] Only a few authors have recommended using these checklists as clinical tools to determine, for example, which individuals with a positive cytogenetic test should undergo a follow-up molecular test (Gringras and Barnicoat, 1998).

Prenatal diagnosis is performed by means of a molecular test on fetal cells obtained by chorionic biopsy or amniocentesis. The molecular test protocol is slightly more complex than for tests performed on blood cells (see Section 3.4.3). Compared with amniocentesis, chorionic biopsy has the advantage of permitting an earlier diagnosis (10th to 12th week of pregnancy), but the sample can be contaminated by maternal cells, which makes interpreting the results more difficult (Maddalena et al., 1994). Furthermore, the methylation process does not always seem to be completed by around the 10th week of pregnancy, with the result that the gene's methylation status cannot, sometimes, be determined with certainty, thus compromising the distinction between large premutations and full mutations. In the event of inconclusive results, amniocentesis is indicated for confirming the gene's methylation status. The possibility of inconclusive results should be mentioned when a chorionic biopsy is proposed to a couple.

In prenatal screening for fragile X syndrome, the main problem for genetic counselling is due to the fact that, with the tests and the current knowledge, it is impossible to predict the presence and severity of mental retardation in female fetuses carrying a full mutation. The risk of mental retardation in these fetuses is about 55%, which makes the decision to continue or terminate the pregnancy a very difficult one[120]. The decision by couples to continue or terminate a pregnancy is based on different factors ranging from the severity of the disease to the anticipated quality of life for the child, themselves and their family. Depending on their personal and cultural backgrounds and their experience of the disease in their family[121], not all couples concerned will assess the situation in the same manner. They should be allowed to make their decision in the most informed manner possible and without any constraints, whether the decision is to terminate or continue the pregnancy. It therefore goes without saying that the private nature of these decisions requires a nondirective approach to the reproductive choices by couples who seek counselling. However, practical experience shows that most couples faced with this situation will opt to terminate the pregnancy. Murray et al. (1997) identified 47 prenatal diagnoses revealing a full mutation in a female fetus in seven published studies. Of those whose outcome was mentioned, the parents chose to terminate the pregnancy in 64% (27/42) of the cases[122].

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[118] It will be recalled that the probability of a woman with a full mutation transmitting it is 50% for each pregnancy. In the case of a premutation, one must also take into account the probability of it expanding into a full mutation, which probability increases with the size of the premutation. A better knowledge of the meiotic instability of premutated alleles could lead to a more accurate estimate of the risk of these alleles expanding into full mutations and therefore of the risk of a woman having an affected child. In the meantime, the risk of transmitting a full mutation to one's offspring justifies prenatal diagnosis for any woman with a premutation (Rousseau et al., 1994), since the expansion risk is not insignificant, even for alleles with only 60 to 69 triplets.

[119] Three cases of transmission of a full mutation by a normal transmitting male have been reported in the literature, in the form of abstracts (Brown et al., 1995; Ventura et al., 1999; Lazarou et al., 1999). In addition, Bridge reports the father-daughter transmission of a full mutation in three unrelated normal transmitting males with a premutation (Bridge et al., 1999; Peter Bridge, Alberta Children's Hospital, personal communication, 2000). Whatever the mechanism involved, Brown et al. (1995) reported the existence of an allelic mosaic in the cerebral cortex. The description of transmissions of full mutations by apparently healthy males carrying premutations changes the risk data governing genetic counselling and raises the issue that prenatal diagnosis for couples where the man is a premutation carrier might be indicated.

[120] Before the molecular test was developed, parents were faced with a similar dilemma, for it was estimated that about 1 girl in 3 exhibited manifestations of mental retardation, even though the cytogenetic test was unreliable in girls.

[121] The study by Curtis et al. (1994) showed that reproductive decision making was not influenced solely by the degree of risk in question. The experience of having an affected child was also an important factor. Conversations with these women revealed that obtaining more information about the behavioural problems associated with fragile X syndrome would have made for a more enlightened decision as to whether or not to have other affected children (couples often want a second child before these problems manifest in the first one).

Other prenatal screening results can pose problems for genetic counselling. Although the intellectual potential of fetuses with allelic or methylation mosaics might, on average, be higher than that of carriers of a full mutation, most are mentally retarded. A better knowledge of the phenotype/genotype correlation will, perhaps, in the future, enable geneticists to refine the information to be given to parents. Lastly, the discovery of a premutation does not have any clinical consequences for the fetus itself and does not generally affect the course of the pregnancy. However, this information will be useful at a later time and should therefore be given to the individual concerned in a timely fashion, which raises, once again, ethical and organizational issues concerning the management of this information.

Recap

Prenatal diagnosis for fragile X syndrome is a practice incorporated into the familial approach and is governed by guidelines (ACMG, 1994; ACOG, 1996). However, it could also be part of other types of strategies. Although considerable advances have been made thanks to molecular testing, as there are fewer errors with genotypic diagnosis than with cytogenetic analysis, the practice of prenatal diagnosis is fraught with residual uncertainties related to the syndrome's phenotypic expression. It is a delicate and complex type of diagnosis, both from the standpoint of performing and interpreting the tests and the ensuing choices made by the couples concerned. Current research on the mechanisms underlying the syndrome's phenotypic expression will probably lead to a refinement in the prognosis. For now, the main dilemma with prenatal screening is not being able to determine the severity of the phenotype in female fetuses with a full mutation. Prenatal diagnosis should therefore be performed within the framework of genetic counselling provided by a team that is experienced in this matter. To document the acceptability of prenatal screening and provide genetic counsellors with tools for better assessment of the informational and support needs of the people concerned, it would be desirable for the psychosocial impact of prenatal diagnosis to be better evidenced.

6.1.3 Proactive diagnosis and screening of high-risk populations

To identify a larger number of affected individuals, one can also employ proactive high-risk population diagnostic and screening strategies. However, this approach requires a more convincing demonstration of the benefits and involves a greater risk of stigmatization and even of discrimination of affected individuals and their families. Although such proactive strategies have not been used in Québec, considerable experience in this regard has been reported in the literature since the early 1980s. The objectives and mechanics of these strategies have changed over time with knowledge and the technical means.

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[122] As for more recent publications, in Ryynänen et al.'s study (1999), the pregnancy was continued in the two cases of female fetuses with a full mutation, whereas in the study by Pesso et al. (2000), the parents of the five female fetuses with a full mutation decided to terminate the pregnancy.

In the early days, the studies were conducted mainly in adults or children institutionalized for mental retardation, in order to determine the frequency of the syndrome among the mentally retarded. A large percentage of these studies began when only cytogenetic analysis was available. Murray et al. (1997) identified ten studies[123] carried out in Europe, the United States and Japan in males institutionalized for mental retardation, but other studies in institutions have been published since then (Holden et al., 1995b; O'Dwyer et al., 1997). The main indication used in these first studies was mental retardation. As a result, most of the diagnoses were made late.

Toward the late 1980s, other objectives, both scientific and clinical, were pursued. Researchers attempted to determine the prevalence of the syndrome on a regional basis. To this end, they no longer targetted only the population institutionalized for mental retardation, but also a perhaps less severely affected population using various points of service, such as residential services for the intellectually impaired, sheltered workshops and day centres (de Vries et al., 1997; Gabarron et al., 1992; Nolin et al., 1991, 1992; Turner et al., 1986, 1992; Webb et al., 1986), as well as children in special schools or special classes (de Vries et al., 1997; Gabarron et al., 1992; Milà et al., 1997; Turner et al., 1986, 1992; Webb et al., 1986). A few researchers have used registers (Kähkönen, 1987) or survey data (Gustafson et al., 1986; Tranebjaerg et al., 1994) to identify high-risk individuals. Also, several studies have evolved into essentially service-type programs or pilot projects. The benefits of case-finding became all the more tangible because the development of linkage analysis paved the way to more reliable cascade screening.

Because there was a desire to make an earlier and more complete diagnosis, the diagnostic and screening indications were broadened to include children with less suggestive clinical signs, such as developmental delay, speech delay, attention disorders, autistic tendencies and learning disabilities (Jacobs et al., 1993; Tranebjaerg et al., 1994; Hagerman et al., 1994b; Slaney et al., 1995; Meadows et al., 1996; Murray et al., 1996; Crawford et al., 1999; Youings et al., 2000). The purpose of broadening the indications is to identify a larger proportion of affected individuals, but it means evaluating a much larger number of children, and recruiting them can be more difficult if they are scattered in regular schools or classes. As a result, this approach requires significantly more resources (both for performing the tests and for genetic counselling). Since 1992, molecular testing gradually supplanted cytogenic analysis and linkage analysis. Even if these tests are faster and more reliable, they are not well suited for very wide-scale use. These logistical problems have prompted some researchers to enrich their sample by prescreening individuals using additional clinical criteria in the form of checklists (Mandel et al., 1994). However, it seems that the use of these prescreening criteria for improving screening yields has not achieved consensus (see Section 3.3.4).

Experience accumulated during this second phase is presented briefly in Appendix VI in the form of tables comparing the inclusion criteria, the selection process for the population of interest, the type of project and the objective or objectives. Most of the authors basically report the uptake rate achieved, the test results and the prevalence estimates based on them. The uptake rates depend on the recruitment methods and the age of the target population and vary according to the cultural context[124]. The diversity of the population selection procedures should be taken into account when comparing the test positivity rates between the different studies. For example, the term "learning disabilities" often refers less to a clinical entity than to an administrative definition of children with special educational needs. This definition therefore depends both on the cultural context and the manner in which the services are organized. In Québec's educational system, this term refers to students with academic difficulties but who are not mentally impaired (see Appendix V). However, it is used in other countries to designate all children with special educational needs, including those who are mentally retarded or with attention disorders. The criteria and the method of identifying these children may therefore differ significantly from one location to another[125]. We are also witnessing a shift in the nosologic designations and classifications, the objective being to prevent stigmatization (Baroff, 1999). However, in certain circumstances, these changes can have an impact on the obtaining of services.

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[123] The test positivity rate reported in these ten studies ranged from 2 to 16%, but in only two of them (with yields of 3 and 4%) were the results of cytogenetic analysis checked with molecular tests. Murray et al. (1997) estimate that, on average, 6% of mentally retarded males have fragile X syndrome (based on a simple, nonweighted mean).

Although these studies have evolved toward the pursuit of clinical objectives, the published accounts do not include an evaluation of all of the possible objectives. The impact of genetic counselling on families is examined in depth only in the more extensive programs, and, to our knowledge, no study has assessed the impact of the diagnosis on the management and prognosis of identified individuals.

Of the more extensive programs whose impact has been assessed in greater depth, the Australian and Dutch programs are particularly noteworthy. The Australian program began in 1984 with the screening of mentally retarded individuals in institutions, residences, special schools and sheltered workshops, then in the entire state of New South Wales (Turner et al., 1986). Subsequently, all intellectually impaired children were screened upon entry into the school system (Turner et al., 1992). It was a large-scale project, considering that by 1997, 3,862 individuals had been tested and 245 affected individuals[126] identified in 225 families, or 1.7% of the 14,225 mentally retarded individuals counted. The authors estimated that 74% of the affected males[127] were identified throughout a state with a population of about 6 million (Turner et al., 1997). On the whole, this program, which is supported by the public authorities, has been well received by institutions and schools. The uptake rate is high, given that 79% of the families contacted between 1984 and 1990 agreed to have their mentally retarded children screened (Turner et al., 1992). The project readily included genetic counselling for the families and the provision of cascade screening. The acceptability of the strategy used was evidenced by a survey among the 90 affected families who took part in genetic counselling[128]. Also, the impact of genetic counselling on reproductive decisions and on prevalence has been examined in depth (Turner et al., 1997; Robinson et al., 1997) (see Section 6.2.1).

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[124] Seveal European studies have achieved uptake rates of close to 60 to 70% (de Vries et al., 1997; Murray et al., 1996; Slaney et al., 1995; Webb et al., 1986; Youings et al., 2000), while in the United States, these rates are more around 45 to 60% (Crawford et al., 1999; Hagerman et al., 1994b).

[125] The proportion of mentally retarded children placed under the heading of "learning disabilities" varies from 15% in the United States (Crawford et al., 1999), 33% in Wessex, Great Britain (reported by Crawford 1999, according to Jacobs et al., 1993), to 55% in Denmark (Tranebjaerg et al., 1994).

[126] For 70% of them, a diagnosis had not been previously made.

[127] The authors explain this by the fact that they did not screen any preschool-age children or children in private schools, which are attended by a third of children.

[128] Most (83%) of these families, in whom an affected individual had been diagnosed, would like to be able to receive genetic counselling if comparable circumstances were to occur.

A regional diagnostic and screening program was instituted in southwestern Netherlands in 1992 (de Vries et al., 1997; de Vries, 1998d), the target population consisting of mentally retarded adults and children in institutions and those attending special schools. By 1997, with an uptake rate of 70%, 1,531 high-risk individuals had been tested. Among them, 11 new cases of the syndrome were found. Based on the total number of cases identified countrywide, the authors estimate that fewer than 50% of the affected males have been diagnosed. The program's acceptability was studied by means of a survey among a large number of participating and nonparticipating families[129] (de Vries et al., 1997; de Vries, 1998a). Among the participating families, the reasons for taking part in the screening and the expectations thereof were realistic[130], whereas the main reason (44%) for not participating was the false belief that an etiological diagnosis of the mental retardation had already been made. Most of the participants considered the strategy acceptable and were open to information being shared with other family members[131]. In the Netherlands, cascade screening is much less advanced than in Australia, since the program is much younger (van Rijn et al., 1997) (see Section 6.2.1).

Recap

Over time, experience with the proactive diagnosis and screening of symptomatic individuals suggests that this approach could, at first sight, be seen as an extension of clinical practice insofar as similar diagnostic indications can be used in sectors where the concentration of potentially affected individuals is higher. However, the proactive nature of these strategies and the fact that they also lead to cascade screening give them the status of screening and require that, for each indication considered, a more rigorous assessment be performed before they are implemented.

Although the experience is relatively extensive and varied, an overall assessment of strategies of this type is not easy. For one thing, the definition of the target populations is not uniform, which makes comparing the various studies from the standpoint of yield a difficult task. Second, the studies have not necessarily examined all the benefits and risks that should be taken into consideration in an exhaustive utility analysis. Lastly, the more extensive programs, which have clinical objectives, have involved a limited number of indications, basically, mental retardation.

These programs nonetheless show that diagnosing and screening mentally retarded individuals is generally well accepted by the families and health professionals involved, although one can expect cultural differences here. Also, these experiences have made it possible to identify a larger proportion of affected individuals than the clinical practice of acting on requests by families. In any event, implementing strategies of this type relies on well-defined places or procedures for selecting at-risk individuals.

6.1.4 Neonatal screening

Neonatal screening is a well-established practice for certain hereditary diseases, such as phenylketonuria, hypothyroidism and tyrosinemia, for which treatment can be initiated early. As for fragile X syndrome, the systematic nature of neonatal screening would, in principle, make it possible to identify a larger number of affected individuals, including sporadic cases, than strategies based on signs or symptoms. The usefulness of this approach has been discussed, where the other strategies have been used extensively and reached their limits.

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[129] 860 families participated in the survey prior to genetic counselling and 681 after, and 153 families who did not take part in screening participated in the survey.

[130] However, unlike the parents of institutionalized children, those of schoolchildren expected the diagnosis to help them obtain better services for their child.

[131] Of the respondents, 80% would recommend the program to others, and 87% had already informed certain relatives of the test results (negative). All seven newly identified families that received genetic counselling had informed the members of their families (de Vries, 1998a).

Unlike the existing neonatal genetic screening programs, one cannot expect, in the case of fragile X syndrome, a direct medical benefit for the child, since the benefit of presymptomatic management for an affected individual has not been demonstrated and since there is no curative treatment on the horizon. Yet, the possibility of therapeutic intervention is the main justification for neonatal genetic screening. Furthermore, it is difficult to compare the potential psychological benefits, for the parents, of identifying the syndrome in a newborn with the benefits anticipated when the syndrome is identified, after consultation, in a symptomatic child. At the presymptomatic stage, screening may be a source of anxiety rather than a solution to a problem perceived by the parents. Furthermore, such a practice could falsely reassure the parents that there will be no mental retardation, which can nonetheless occur in 1 to 3% of children. For now, the benefit of neonatal screening would be to diagnose fragile X syndrome very early, even in the absence of a family history, which would permit timely genetic counselling for the nuclear family (Fryns, 1995; Mandel et al., 1994).

Neonatal screening raises another ethical issue because the molecular test not only identifies full mutations, but premutations as well. Even if newborns with a premutation can benefit from this information when the time comes for them to have children, the absence of immediate benefits goes against the established principles justifying the screening of children. This also raises ethical and organizational problems concerning the storing and communication of genetic information.

Instituting neonatal screening requires adapting the molecular tests for wide-scale use, an area of research that is very active at this time (Dawson et al., 1995; Holden et al., 1995a; Hong et al., 1999; Strelnikov et al., 1999). One of the new avenues being explored is a test based on anti-FMRP antibodies that could eventually overcome the problem of identifying premutations (Willemsen et al., 1995, 1997, 1999). However, this technique raises other problems, since, for now, this method only identifies individuals in whom FMRP production is completely inhibited. Consequently, it is limited to the identification of boys with a full mutation and does not permit identification of boys with mosaics or girls with a full mutation. Their families could therefore not obtain genetic counselling. Although research is mainly focusing on the tests' technical development, no experience concerning the implementation of such a strategy has yet to be found in the literature[132].

In short, despite the possibilities offered by the existing infrastructure, neonatal screening of fragile X syndrome cannot be considered at this time because of technical difficulties and ethical considerations which are due largely to the limits of our knowledge. Of course, it is not impossible that this situation will change in the future with the development of tests that target affected individuals more specifically, together with new therapies or the possible discovery of other benefits associated with management from birth.

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[132] The results of a pilot study involving 1,000 male newborns were reported in the literature, but the study was carried out anonymously for the purpose of determining the prevalence of normal, premutated and mutated alleles in the general population (Holden et al., 1995a).

Table 6.2: Strategies for diagnosing and screening for affected individuals: main features, experience and brief assessment

	Features	Experience	Assessment
Clinical practice	• On demand • Prescreening based on: - Recruitment channels - Signs for referral	• Considerable experience with regard to mental retardation • Practices vary with regard to the other diagnostic indications • Limited evaluation	• Practice not centralized (numerous points of entry) and not systematized (referral, workup for mental retardation and developmental delay, screening for mental retardation and language acquisition delay, diagnostic indications) • Underdiagnosis and late and selective diagnosis (diagnosis: ♂ > ♀, + if family history)
Prenatal diagnosis	• Indication based on the parents' genotypic status	• Experience scattered • Assessment of psychosocial consequences very incomplete	• Ethical problem associated with predicting phenotype in ♀: dilemma for parents and problems for genetic counselling • Technical problems if chorionic biopsy
Proactive screening	• Methods vary according to: - Target population and institution - Diagnostic indications - Prescreening criteria • Research projects and public programs • Individual follow-up and ± intensive cascade screening	• Experience quite vast (see Appendix VI) • In-depth evaluation of a few programs	• Feasibility depends on the manner in which the services are organized • Uptake and acceptability generally good but may depend on cultural factors
Neonatal screening	• Existing infrastructure	• No published experience	• Ethical problems (prognosis not improved; identification of premutations) • Technical problems (tests, resources)

6.2 CARRIER SCREENING STRATEGIES

The strategies used or proposed for identifying asymptomatic mutation carriers (full mutations or premutations) include **cascade screening**, the **screening of women with a family history of mental retardation or other signs**, the **screening of pregnant women** with no particular family history, and the **preconceptional screening** of women of child-bearing potential. Cascade screening and the screening of women with a family history of mental retardation are part of current clinical practice, which constitutes an on-demand strategy. The screening of pregnant women with no particular family history and the preconceptional screening of women of child-bearing potential depend on more proactive, though not always systematic, recruitment.

6.2.1 Cascade screening

Cascade screening is intended for all couples of child-bearing potential at risk for transmitting fragile X syndrome because of a family history of it[133] and possibly for other relatives. This type of screening is an adjunct to the different case-finding strategies mentioned above or to the carrier screening strategies discussed below. In current clinical practice, cascade screening is performed after a symptomatic individual is diagnosed with the syndrome. It is done, within the context of genetic counselling, by proceeding systematically from the index case in order to determine the carrier status of the members of the family—the nuclear one first, then the extended family—who agree to this. Usually, contact with the relatives is made through the affected individual's parents, a practice that is based on respect for the autonomy and confidentiality of the members of the affected family and of the relatives. Actually, a more proactive approach to recruiting relatives would raise ethical problems and could, in some cases, violate professional deontology.

In cascade screening, the carrier status is usually determined in the women of child-bearing potential, given that those with a full mutation or a premutation are at risk for having affected children. The usefulness of molecular testing in apparently healthy males has been mentioned by certain authors (Rousseau, 1994). Previously, there did not seem to be any immediate benefit in determining the carrier status of normal transmitting males[134], but this situation may have changed in light of more thorough knowledge that has been acquired with regard to high-functioning males (see Section 2.4) and in light of the recent description of rare cases of normal transmitting males (NTMs) transmitting full mutations to their daughters (see Section 6.1.2). Also, not confirming the carrier status makes managing the information for future generations more difficult. A method of passing on the information must then be worked out so that it is received in a timely manner by daughters, of NTMs, who are at risk for having affected children[135].

A systematic assessment has not been done of cascade screening, although a few authors do report their clinical experience (Cossée et al., 1997; Ryynänen et al., 1995; van Rijn et al., 1997). Also, certain studies involving proactive diagnosis in mentally retarded individuals followed by cascade screening provide data on the families' uptake rate[136], the proportion of relatives who underwent testing, and the impact of cascade screening on reproductive decisions (Robinson et al., 1996; Turner et al., 1992, 1997; van Rijn et al., 1997).

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[133] Ever since an association was established between premature ovarian failure and positive premutation carrier status, cascade screening has been applied as well to women with this new indication and to their relatives.

[134] It will be recalled that normal transmitting males have a premutation that they will transmit to all of their daughters. The latter will not be symptomatic but may transmit the syndrome to their children if the premutation expands into a full mutation.

[135] The long-term effect of a wide-scale screening program, such as the one in Australia, on the birth prevalence of the syndrome might depend, to a certain extent, on the identification of NTMs and on the transmission of the information to the following generation (Robinson et al., 1996).

Cossée et al. (1997) report that 85% of the 44 families detected following the diagnosis of index cases in clinics participated in genetic counselling, which led to the identification of 98 new full mutation (FM) or premutation (PM) carriers (or 2.2 per family)[137] and to 9 prenatal diagnoses. Ryynänen et al. (1995) described the follow-up of 59 families in genetic counselling[138]. 48.1% of the at-risk members underwent molecular testing, which led to the identification of 235 FM and PM carriers (4 per family) and 10 affected fetuses out of 21 pregnancies. Van Rijn et al. (1997) followed, for a period of at least one year, 19 families, some of whom had been referred to them, while others had been identified by proactive institutional and school screening. They showed that the effectiveness of transmitting information within families and participation in molecular testing are inversely associated with the degree of relationship[139]. Cascade screening nonetheless led to the identification of 56 FM or PM carriers (3 per family).

The information provided by the Australian diagnostic and screening program reflects the results of more than 10 years of cascade screening and is indicative of the vigorousness of the follow-up provided to the families[140]. By December 1996, 225 families had been identified and 2,337 relatives tested. Apart from the 886 affected individuals (an average of 4 per family) in whom a full mutation had been documented, 532 asymptomatic FM or PM carriers (including 103 normal transmitting males) were identified, or 2.3 per family[141] (Turner et al., 1997). In ten years, 104 asymptomatic women carriers opted for prenatal screening, which led to the identification of 19 male fetuses with a full mutation.

Toward the end of the 1980s, a case-control study was undertaken to document the impact of genetic counselling (only cytogenetic analysis was available at the time) on reproductive decisions by women who were first-degree relatives of an index case. A 26% reduction in births was documented (77 vs. 104) among the couples that received genetic counselling, but this reduction was attributable to the women with normal intellectual capacity only. Among the women who subsequently became pregnant, 61% underwent prenatal diagnosis (Turner et al., 1992). There are still some unfollowed pregnancies, usually in mentally retarded women, but, in the early 1990s, the proportion of couples opting for PND increased to 77% and, for couples who already had an affected boy, to 91% (Robinson et al., 1996). This increase in the use of PND since molecular testing became available and the fact that 79 nonaffected pregnancies had been carried to term indicated to the authors that we are witnessing renewed confidence, on the part of affected families, in their reproductive potential.

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[136] The first programs aimed at providing services to families only reported the number of families that took up the offer of genetic counselling: 50% for Nolin et al. (1992) and 61% for Gabarron et al. (1992).

[137] Between 1991 and 1996, the criteria established by the Association française pour la prévention des handicaps de l'enfant (AFDPHE for reimbursing the molecular diagnosis of fragile X syndrome) implied that mentally retarded individuals would be diagnosed only for the benefit of a female relative of child-bearing potential who might request genetic counselling (Cossée et al., 1997; Mandel et al., 1996).

[138] These families were identified from different sources: mentally retarded individuals or individuals with language acquisition delay referred for diagnosis, pregnant women with a family history of mental retardation requesting genetic counselling, and families contacted based on a cytogenetic register (Ryynänen et al., 1994, 1995). The latter approach could involve proactive recruitment of the families concerned, which differs from the most common approach in medical genetics but which could explain, in part, the results obtained. Because of a lack of information, we will not discuss this particular proactive form of cascade screening.

[139] Overall, 34% of the relatives were informed of the familial risk (100%, 59%, 39% and 3% for the first-, second-, third- and fourth-degree relatives, respectively), and 26% of the relatives underwent molecular testing (90%, 37% and 30% for the first-, second- and third-degree relatives, respectively). However, it should be noted that families in the Netherlands are especially mobile, given that one half of the relatives who were not informed were living abroad.

[140] Each year, an information leaflet is sent to all the families recorded in a register since the early 1990s, and a re-evaluation by means of molecular testing was proposed to all the families which had been previously identified.

[141] The families' participation in cascade screening nonetheless varied considerably, given that in 40% of them only the first-degree relatives took part.

Lastly, controlling the number of pregnancies and the selective termination of pregnancies involving affected fetuses have led to a decrease in the birth prevalence of the syndrome in these families[142] and in the statewide prevalence, given that about three quarters of the families are apparently now known, because of the program (Robinson et al., 1996; Turner et al., 1997). In New South Wales, it is reported that the birth prevalence for boys has decreased from 1/4,000 to 1/10,000 and that more than 60% of affected children are now born in one fourth of the families that have not been etiologically diagnosed. The performance of the current strategy for diagnosing and screening for affected individuals in schools followed by cascade screening may, however, well decrease because the syndrome is better known in the medical and school sectors and because potentially affected individuals are referred more and more to genetic specialists. In Australia, researchers are therefore starting to consider the use of alternative strategies, such as screening pregnant women or neonatal screening (Turner et al., 1997) (see discussion in Section 6.3).

The efficacy of cascade screening therefore basically depends on the dissemination of information within the families and on the acceptability of the available services. However, for fragile X syndrome, communication problems within affected families have been brought to the fore. These problems are due mainly to the guilt associated with the hereditary transmission of mental retardation and to the possibility of several members of the family suffering from mental impairment (see Section 7.1.2). The methods used to facilitate and maintain contact with the families have an impact on their participation and satisfaction. Furthermore, according to a simulation study and based on the percentage of carriers in a given population that can be identified by cascade screening, such screening is not very effective, with the result that the proportion of carriers who might receive genetic counselling is low (Wildhagen et al., 1999).

Recap

Cascade screening enables one to determine the genotypic status of symptomatic relatives, but its main objective is to identify asymptomatic individuals at risk for transmitting the mutation to their offspring. Cascade screening is part of current medical genetic practice when a diagnosis is made in an index case referred to a clinic, but it could be performed further to any type of strategy. It cannot be separated from genetic counselling, which provides both the necessary information and support for families to deal with the new risk data given to them and to make informed reproductive decisions.

The proportion of carriers who can be identified by this type of screening depends on the case-finding method and on the percentage of family members who participate in genetic counselling and who wish to be tested. The uptake rate varies with the intensity of the follow-up of the families but also depends on how effectively information is communicated within the family.

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[142] As with any other carrier screening strategy, this approach does not in any way change the prevalence of premutation carriers.

The experience accumulated worldwide in this regard tends to show that the practice of cascade screening is generally well accepted, that it meets a need on the part of the families concerned and that, on the whole, it has an impact on their reproductive decisions. In Australia, where this approach has been used systematically for more than ten years, there has been renewed confidence, on the part of affected families, in their reproductive potential and a decrease in the prevalence of the disease.

6.2.2 Screening of women with a family history of mental retardation or other signs

Under the guidelines issued by the American College of Medical Genetics (ACMG) and the American College of Obstetricians and Gynecologists (ACOG), it is advisable to suggest to a woman of child-bearing potential with a family history of mental retardation that she undergo a test to determine her carrier status. Although this practice has not been systematically evaluated, a few genetic centres report their experience in this area and the number of fragile X families identified this way[143] (Brown et al., 1996; Kaplan et al., 1994; Wenstrom et al., 1999).

The issue of broadening the indications also comes up in the context of carrier screening. A few genetic centres have, in fact, proposed carrier screening to pregnant women with a family history of developmental delay, language acquisition delay, learning disabilities, autism or attention disorders, but the available data are still limited[144] (Kaplan et al., 1994; Howard-Peebles et al., 1995; Wenstrom et al., 1999).

For some of these indications, such as learning disabilities and attention disorders, the usefulness of this broadened approach will, in the end, depend on still-awaited assessments of the usefulness of molecular testing in children with these symptoms. Also, the performance of this strategy (in terms of the test positivity rate) will probably be less than that of screening index cases themselves (Wenstrom et al., 1999), but it is sometimes impossible to obtain, within the required time, diagnostic confirmation of fragile X syndrome in index cases[145].

Lastly, the recent documentation of an association between premature ovarian failure and positive premutation carrier status would warrant including a family history of premature ovarian failure in the indications for testing if the carrier status has not been directly checked in the at-risk relatives.

In short, determining the carrier status of women of child-bearing potential with a family history of mental retardation or any other recognized diagnostic indication is justified when a diagnosis of fragile X syndrome in the index case cannot be confirmed within the required time. The broadening of the indications should, in clinical practice, be managed on a case-by-case basis until more thorough evaluations have been carried out.

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[143] Brown et al. (1996) detected two FMs and four PMs in 344 pregnant women with a family history of mental retardation, and Kaplan et al. (1994) found one PM in 126 pregnant women with such a history.

[144] Kaplan et al. (1994) did not find any mutations in 46 women with a family history of developmental delay, language acquisition delay or autism, while Howard-Peebles et al. (1995) identified four affected families from 231 pregnant women with a family history of mental retardation, developmental delay, language acquisition delay, attention disorders or autism. Wenstrom et al. (1999) did not find any premutations or full mutations in 263 women with a family history of mental retardation, attention disorders, learning disabilities or autistic features.

[145] In the study by Howard-Peebles et al. (1995), only 19 women opted to solicit the participation of the affected individual in their family, the others choosing to be tested themselves.

6.2.3 Screening of pregnant women

If one wishes to identify all at-risk families, more systematic strategies that do not involve any clinical selection criteria or documenting a family history will need to be considered. Indeed, the main limitation of carrier screening strategies based on a family history is that sporadic cases cannot be detected.

Since a study carried out in Québec showed that 1 in 259 women is a premutation carrier (Rousseau et al., 1995), the question arose as to whether molecular testing for determining carrier status should be offered systematically as one of the many screening tests offered to pregnant women. A positive result would, in such cases, lead to an offer of genetic counselling, then to prenatal diagnosis, if desired, and lastly, to genetic counselling for the extended family. This strategy would have the advantage of reaching all the women at a time when they are concerned about the health of their offspring and thus, if the uptake rate is high, of identifying a greater number of at-risk pregnancies than the strategies involving cascade screening.

The drawback of this strategy is the brief window of time between the detection of a mutation in a pregnant woman and when crucial decisions have to be made as to whether to undergo prenatal diagnosis and to continue the pregnancy. Effective communication of information is therefore of the utmost importance, as is the nondirectivity of the interventions.

Several authors have come out in favour of implementing this type of screening in the future and state that a number of technical and/or ethical issues need to be resolved or clarified before considering instituting such screening in clinics (Palomaki and Haddow, 1993; Howard-Peebles et al., 1993, 1994; Mandel et al., 1994; Palomaki, 1994; Rousseau et al., 1994; Ryynänen et al., 1995). A debate has begun over these issues and has brought to light other reservations and questions (Bonthron, 1993; Bundey, 1993; Howard-Peebles et al., 1995; Meadows and Sherman, 1996; Barnicoat, 1997; Berry, 1997), several of which bring us back to the issues raised by prenatal diagnosis. Of these, the predominant concern is that the phenotype cannot be predicted in female fetuses with a full mutation, for only 50 to 55% of them will develop mental retardation. A problem more specific to this strategy is that it is not known if the risk of premutation expansion determined for affected families applies in the absence of a family history (Bundey, 1993; Mandel et al., 1994; Oostra and Halley, 1995). Yet, this, together with the prevalence of premutations, is one of the key pieces of information needed to assess the usefulness of such an approach. A large study comparing these risks in the presence and in the absence of a family history is currently underway in Québec. Lastly, a prerequisite for instituting these strategies is developing and validating analytical methods better suited to large-scale screening.

Murray et al. (1997) found that few data were available for assessing the feasibility of a systematic screening program for pregnant women. In a pilot project instituted at a prenatal diagnosis centre in late 1993 in Fairfax, United States, women referred to the centre (mainly for advanced maternal age) were invited to undergo, at their expense, fragile X molecular testing[146] (Howard-Peebles et al., 1995; Spence et al., 1996). However, the population was selected not only because the participants had already been referred for genetic counselling in general due to advanced maternal age, but also because of self-selection based on their history, given that nearly one third of them had a family history of mental retardation, learning disabilities, autism or attention disorders[147].

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[146] The fact that the test was not free no doubt considerably affected the uptake rate, which was 21% (688/3,345). Consequently, this figure does not necessarily apply to Québec.

More recently, a Finnish study (Ryynänen et al., 1999) involving more than 1,500 pregnant women examined the feasibility and acceptability of a screening program for pregnant women. Based on the 85% uptake rate for the test[148], the fact that prenatal diagnosis was performed in the 18 women with more than 50 CGG repeats[149], and the results of the survey among the participants[150], the authors conclude that screening for fragile X syndrome would be well accepted if incorporated into prenatal care in the Finnish health-care system. Although the results, in terms of feasibility, of this pilot project might apply to Québec, the acceptability assessment does not seem sufficiently conclusive to us, since it does not deal with questions like the psychological impact of a diagnosis in the absence of a family history and the difficulties associated with reproductive choices made in such circumstances.

Two screening programs were instituted in Israel in 1992 and 1994 in which women could opt for molecular testing, for which there was a charge, as part of prenatal follow-up. They were large-scale programs, but since the results of one of them was published only in the form of an abstract, it will really not be possible to assess its impact[151] until a more detailed report is published (Falik-Zaccai et al., 1999). In the other program (Pesso et al., 2000), 9,459 women with no family history of the syndrome opted for the test, either prenatally (80%) or preconceptionally (20%). However, about 11% of them reported a family history of mental retardation or other developmental problems[152]. Apart from the prenatal screening rate[153], the authors do not seem to have examined other aspects of the screening program's psychological impact or acceptability. Although an uptake rate applicable to the entire population cannot be estimated from this study[154], the authors recommend that screening be extended to all pregnant women in the country and be available on request prior to conception.

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[147] Three premutations were found in the 745 participants (including those tested because of an egg donation) who did not have a family history of mental retardation or other signs, which works out to a prevalence of 1/248 (based on premutations with more than 60 triplets).

[148] Of the 1,477 women who opted for the test, six had a premutation with more than 60 triplets (which indicated to the authors that the prevalence of premutations in their population was 1/246), but 12 other women had between 50 and 60 triplets.

[149] Furthermore, in the 43 women with between 40 and 50 triplets, six requested prenatal diagnosis.

[150] The survey was completed by 16 of the 18 women with more than 50 triplets and by 33 (out of 54) women with no expansions. It was found that the participants did not feel any coercion or pressure on the part of family and friends or the professionals to undergo the test, but that half of the respondents would have wanted to receive more information about the syndrome and the significance of having positive carrier status.

[151] When the abstract was published, 404 women with a family history of mental retardation and 9,426 with no family history of mental retardation or learning disabilities had undergone the test. In the first group, seven premutations (PMs) (more than 52 triplets) and one full mutation (FM) were found, as opposed to 111 PMs and three FMs in the second group. All the carrier women accepted the offer of prenatal screening. The authors conclude that the prevalence is high (one FM in 3,145 women and one PM with more than 52 triplets in 85 women with no particular history), which, in their opinion, could justify instituting a nationwide screening program (Falik-Zaccai et al., 1999).

[152] In the at-risk group, four PMs with more than 55 triplets and three FMs were detected, while in the women with no particular family history, molecular testing revealed 59 PMs and one FM. In this latter population, the prevalence of premutations with more than 55 triplets therefore seems higher (about 1/145) than that determined for Québec (1/259).

[153] Of the 134 pregnant at-risk women, six declined prenatal diagnosis, but five of them were carriers of fewer than 60 triplets and had been informed of the low risk of expansion that this type of premutation poses.

[154] The participants had been referred to the Institute of Genetics by a physician or had chosen on their own to go there for a consultation. Selection based on history is plausible in this study as well.

Recap

Screening pregnant women is an alternative approach for identifying a larger proportion of potentially affected families, since it permits identification of at-risk couples with no family history before the birth of a first affected child.

Experience accumulated to date is, however, relatively limited. It would be desirable for other studies to thoroughly assess the acceptability of this approach and to clarify the main ethical issues concerning phenotype prediction and information management.

In short, for this approach to be feasible and acceptable, a certain number of technical (validated tests suited for wide-scale screening) and scientific (genotype/phenotype correlation, risk of expansion in the absence of a family history) problems must first be resolved.

6.2.4 Preconceptional screening of all women of child-bearing potential

Like the screening of pregnant women, preconceptional screening would, in principle, make it possible to identify all at-risk families, if such screening is found to be acceptable and if women participate in it. The advantage over screening pregnant women is, of course, that it gives more time to think things through and offers more options during reproductive decisions. However, the limitations of screening pregnant women and the ethical issues that it raises also apply to preconceptional screening: unresolved technical constraints (validated tests suited to wide-scale screening) and weaknesses in predicting phenotype and in assessing the risk of transmission.

If we look at the experience with cystic fibrosis, the preconceptional period would not be the most suitable for instituting a hereditary disease screening program, since there is less motivation and participation in such programs than during pregnancy. Furthermore, from a logistic standpoint, it may be more difficult to reach this population, depending on the target age and the local organization of services (premarital services, family planning services, schools). However, fragile X syndrome differs in nature from cystic fibrosis, and experience with screening other diseases, such as Tay-Sachs (Mitchell et al., 1996), should be taken into account, since data specific to fragile X syndrome are quite limited[155]. In short, caution should be exercised when extrapolating conclusions based on experiences with other hereditary diseases and in other cultural contexts. Also, before considering instituting proactive screening aimed at this population, it should be determined if a well-targetted information campaign would result in their seeking out the appropriate services when a pregnancy is being considered.

In short, preconceptional screening theoretically offers the possibility of identifying all at-risk families but entails logistical problems that affect its feasibility. Experience specifically with fragile X syndrome is very limited, and experience with diseases inherited through autosomal recessive transmission does not, alone, constitute a sufficient reference.

Although preconceptional screening in all women of child-bearing potential offers an undeniable advantage over screening pregnant women from the standpoint of time and reproductive choices, this approach entails additional difficulties concerning the location, the time and the methods to be used to recruit the target population.

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[155] In Pesso et al.'s study (2000), which is described above, about 1,600 participants opted for the test prior to conception, but the impact of the screening in this group in particular cannot be assessed from the current data.

6.3 DISCUSSION

Since there are currently no specific criteria for evaluating screening strategies based on molecular testing, our evaluative approach is based on assessing the utility, feasibility and acceptability of the various proposable strategies. To do this, accumulated international experience must be compared to the regional context, which partly determines their feasibility and acceptability, as well as their utility. In this regard, we often have to rely on incomplete data concerning the utility and acceptability of strategies for which international experience is limited. Moreover, because of the rapid pace at which knowledge and technical capabilities are evolving, the feasibility assessment is subject to change. Having reached the end of this prerequisite exercise, we now present a discussion of three main groups of strategies, starting with those whose implementation is the least plausible at this time: low-risk-population screening, proactive high-risk-population screening and lastly, strategies that are already part of clinical practice.

6.3.1 Low-risk-population screening

For fragile X syndrome, the screening of low-risk populations can assume the form of neonatal screening or the screening of women, either before conception or during pregnancy. Theoretically, these approaches would have the advantage of identifying a larger number of affected individuals, in the case of neonatal screening, or a larger number of at-risk families, in the case of carrier screening. Since the detection rate of such strategies depends directly on the uptake rate, achieving these benefits obviously presupposes proactive recruitment, even if participation is voluntary.

Nonetheless, it is difficult to document the actual benefits and the acceptability of these approaches, given the little experience reported in this regard. The few research projects or pilot projects carried out thus far concerning carrier screening of women do not yet permit a complete assessment. Since we recognize that this could potentially be a very useful approach, it would be desirable to see other pilot projects developed to examine the benefits, risks and main issues underlying its acceptability.

Although it is always possible to incorporate neonatal screening for fragile X syndrome into the existing neonatal screening infrastructure in Québec and although the feasibility of neonatal screening in Québec is presently being studied from a technical standpoint, there is no international experience concerning the instituting of such an approach that could enlighten us about the problems specific to diagnosing a nontreatable condition (and to neonatal carrier screening that the use of certain techniques raises).

These alternatives are mainly mentioned in the literature because of the limitations of the other approaches, which are unsuccessful at identifying all affected individuals or all affected families. The first researchers to seriously consider developing these alternatives came from areas where the other strategies had been used extensively, with a decreasing positivity rate. The acceptability of these approaches also seems to depend on previous regional experience, the approaches being warranted more where information about the syndrome has already been broadly disseminated among health and education professionals and the population.

Table 6.3 Carrier screening strategies: main features, experience and brief assessment

	Characteristics	Experience	Assessment
Cascade screening	• Subsequent to clinical diagnosis or the various screening strategies • Indissociable from genetic counselling	• Widespread experience constituting the usual approach in medical genetics • Assessment limited for the clinical practice of diagnosis but thorough for a few proactive high-risk-population screening strategies	• Participation varies according to the level of contact and the duration of the program • Intrafamilial communication often a problem • Acceptability generally good • Impact on reproductive decisions and on prevalence documented for a program with an intensive follow-up
Screening of ♀ with a family history of mental retardation or other signs	• Indications rare if the diagnosis cannot be confirmed in the index case	• Limited experience • Assessment limited, especially for indications other than mental retardation	
Screening of pregnant ♀	• Mention is made of the possibility of identifying more women carriers, including those with no family history	• Limited experience, partly biased toward a population at risk based on family history	• Scientific problems (risk of expansion, genotype/phenotype correlation) • Ethical problems (♀ phenotype) • Technical problems (tests, resources)
Preconceptional screening	• Theoretical advantage over screening pregnant ♀: - More time to make decision - More reproductive choices	• Experience very limited	• Problems comparable to those of screening pregnant ♀, plus: - Logistical problem (recruitment) - Right timing unclear - Participation not secured (motivation ?)

However, there seems to be a consensus in the recent literature that case-finding strategies should be used first before instituting low-risk-population screening strategies, as long as the scientific and technical problems have not been resolved and as long as the ethical issues have not been debated more thoroughly. Neonatal screening cannot be recommended at this time because of the problems associated with identifying premutation carriers and because early management does not confer any benefit. Preconceptional screening and the systematic screening of pregnant women with no particular family history are subject to the limits of our prognostic capabilities. Furthermore, there would need to be validated tests suited for wide-scale screening.

In conclusion, it is still too early to implement these strategies because of unresolved technical and scientific problems and of ethical implications due, in large part, to the limits of our knowledge. The methods and knowledge in this area are evolving at an extremely rapid pace, with the result that, in the future, the situation will have to be reassessed in light of the new developments. The experience acquired with these strategies for fragile X syndrome is limited, and pilot projects are needed in order to rigorously assess the utility, feasibility and acceptability of the proposed strategies.

6.3.2 Proactive screening of high-risk populations with cascade screening

Proactive fragile X screening in high-risk populations has been performed extensively worldwide using various methods. The at-risk individuals have generally been recruited at institutions, outpatient service centres or schools. The efficacy of these programs, i.e. the percentage of affected individuals identified, depends on their feasibility and acceptability and on the target population (depends on the indications used, the prescreening procedures, etc.). The feasibility of this approach clearly depends on the regional organization—and especially the centralization—of the educational services and specialized services offered to these individuals. The constraints associated with the organization of services are all the more problematic because early identification of the syndrome is desired. The acceptability of these programs is generally good, although cultural differences are observed. Apart from case-finding, some programs are aimed at performing cascade screening. The efficacy of such programs, i.e. the percentage of at-risk relatives identified, depends on the amount of time that has elapsed since the program was instituted, on the intensity of the follow-up of the families and on the communication problems frequently encountered in the families.

On the whole, this approach has been considered useful for improving the diagnosis of affected individuals and carrier identification in extended families or for promoting awareness of the syndrome. However, it does have limitations that are now clearly known: these programs end up achieving a decreasing case-finding yield; the screening of the families is often incomplete; and carriers with no family history cannot avail themselves of this service. In all, the proportion of premutation carriers that could be identified with this approach is apparently low, with the result that most couples at risk for transmitting the syndrome cannot receive genetic counselling in a timely fashion (Wildhagen, 1999).

The accumulated experience is quite varied, but most of the major programs that have examined the efficacy and acceptability of this approach have involved a limited number of indications, mainly mental retardation. Few studies have involved the additional objective—now a given—of identifying affected individuals as early as possible. Achieving this objective would require a broadening of the diagnostic indications and the concurrent use of other selection criteria. Yet, despite the accumulated experience, there is no consensus in the literature as to the indications to be used, such as attention disorders and learning disabilities in the absence of mental retardation, or to the use of checklists. If the indications to be used in a proactive screening program are to be consistent with the diagnostic indications acceptable at clinics, then it is probably reasonable to stick to a more limited number until research quantifies the level of risk for each clinical presentation.

Strategies for identifying signs, such as developmental delay[156] and language acquisition delay, are a radically different approach, and, insofar as they could constitute a prerequisite for the diagnostic workup or for screening for the syndrome, would warrant investigation. Although the benefits of the early management of speech problems have been clearly demonstrated, the usefulness of implementing such screening depends on the organization of services and especially on accessibility to speech therapists[157].

In Québec, it seems that the management of children with developmental delay, mental retardation or learning disabilities varies considerably, both in the medical, social and educational sectors. Given the extent, in Québec, of the movement toward social and school integration, identifying at-risk individuals would no doubt require the deployment of more resources than before or than in those countries where such individuals are grouped together more. This being said, a proactive screening strategy in institutions or special classes or schools would probably not identify most of the affected families, unless one waits until the end of elementary school or the beginning of high school, which would not offer any more major advantages than the usual clinical practice. If one wanted to organize earlier screening, a more systematic approach for identifying signs would have to be instituted—in regular schools or in preschools—that could eventually support fragile X screening. However, in Québec, there is presently no structure that could support a systematic effort to screen for developmental delay or speech delay. Furthermore, the acceptability by the people concerned depends on the perception of the benefits, which is closely tied to prior recognition of a problem in the child. Lastly, the social acceptability of a selective, proactive approach is not established in Québec because the population and many health professionals are ill-informed about the syndrome and because of the conceptual differences concerning mental retardation and the communication problems between the educational, social and medical sectors.

In any event, it is not desirable to consider instituting a proactive screening strategy without first having conducted pilot projects involving an in-depth assessment of the feasibility and performance of the proposed strategy in Québec (number of children identified, age at diagnosis, genetic counselling for nuclear and extended families), its psychosocial impact on the families concerned, and the necessary resources.

6.3.3 On-demand clinical practice with cascade screening

Diagnosing symptomatic individuals, cascade screening and prenatal diagnosis are part of the familial approach usually used in medical genetics in Québec and elsewhere. Screening pregnant women with a family history of mental retardation or possibly other indications is an extension of this approach and is done when a diagnosis cannot first be made in a symptomatic relative.

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[156] There exist several tools for screening for mental retardation, but there is no general agreement as to the most effective approach (Cooley, 1999, Filipek et al., 2000).

[157] A recent British study that involved a literature review and a meta-analysis of the available data assessed the usefulness of universal screening for speech and language delays (Law et al., 1998). The benefits of the early management of speech problems was clearly demonstrated. Despite this, the authors believe that, for Great Britain, universal screening is not required because speech therapists are well integrated into first-line care services and because identifying problems and providing referrals to services do not pose a problem. In Québec, however, accessibility to speech therapists seems to be a major problem.

This practice meets a need on the part of the families, since it permits clarification of the diagnosis in symptomatic individuals and enables the families concerned to obtain genetic counselling. Although it is a widespread practice, few assessments of its efficacy can be found in the literature. It is nonetheless agreed that it has definite utility, but a few articles suggest that, if the quality of the service is not up to par, then a negative impact on the utility and acceptability of this practice should be expected. For example, we note that the time it takes to make a diagnosis, the psychosocial support provided when the diagnosis is communicated, timely access to genetic counselling and referrals to the necessary support and integration services vary enormously, which can create obstacles to obtaining potential benefits and result in dissatisfaction with the needs response. Also, because of the limits of our present knowledge, it is not certain that the tests are useful for certain indications, since justification for the test is marred by a lack of convincing data on the level of risk associated with each potential indication.

As a diagnostic and screening strategy, this clinical practice has its limitations as well, some of which are due to the organizational modalities, while others are inherent in the nature of the syndrome. Because of the variability and insidious occurrence of the symptoms and because of the selection procedures—which are invariably carried out in a system that responds to requests by families and which is based on the referral of these families to test orderers—, fragile X syndrome is underdiagnosed. The complexity of genetic counselling and the emotional burden associated with the diagnosis hinders the dissemination of information within families, thus limiting the efficacy of cascade screening.

Also in Québec, the syndrome seems to be underdiagnosed, given that there is a large gap between the number of individuals with a diagnosis confirmed by molecular testing and the expected number of affected individuals in the population, based on prevalence data for the syndrome (see Section 5.1). The proportion of mentally retarded children who have been tested is probably very low as well, assuming that about 1 to 3% of school-age children are mentally retarded. There is a gradual downward trend in the age at which the tests are being ordered, but the diagnosis is still often made late. Since the interval between the first two pregnancies is less than 36 months in practically 55% of Québec families[158], the decrease in the age at diagnosis will not succeed in totally eliminating the recurrence of the syndrome in the families concerned. Also, a number of clinicians note that in Québec as well, genetic counselling and cascade screening are far from having reached all the members of those families that have already been identified.

An examination of the situation in Québec has provided several indications concerning the factors that might be contributing to the diagnostic confirmation being incomplete (and possibly random). The relative lack of knowledge of this clinical entity by first-line health professionals, the numerous points of entry into the system, the lack of collaboration between the health-care system and the school and preschool system, the absence of a standardized workup for developmental delay and mental retardation, and the lack of Canadian guidelines for ordering molecular tests lead to quite variable recruitment channels, unplanned prescreening, and a "prediagnostic" path that is described by parents as being difficult and painful.

Having reviewed the situation in Québec and the international experiences in this area, we note that accessibility to services and the coordination of diagnostic and management services are the main weak points of this practice. Although we are not able to provide any ready-made solutions, we can make a few recommendations aimed at encouraging the parties concerned to assume more responsibility and at stimulating intersectorial cooperation. To rectify the deficiencies of this clinical practice, various measures aimed at professional practices, but also at the organization of services, could be considered.

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[158] According to 1996 Québec statistics (written personal communication, Bureau de la Statisitque du Québec, 1998).

From the standpoint of professional practices, the efforts, which must be agreed upon in order to improve case-finding, should be part of an approach aimed at improving the diagnostic workup for all developmentally delayed or mentally retarded children[159] or even those with language acquisition delay. Fragile X testing should therefore not be dissociated from the other clinical examinations and diagnostic tests necessary in this regard. Developing Canadian guidelines for molecular testing might be useful, but preparing and disseminating a standardized workup for developmental delay and mental retardation would be especially so. Such a workup, which would be particularly useful in first-line services, might assume the form of an algorithm showing the tests and examinations to be performed first and precise indications for referring the individual to superspecialized services (genetics, neuropediatrics or others, depending on the indication)[160]. An extension of this approach would be to also include signs that could serve as a guide for referrals by reeducation professionals and professionals in the day-care, school and preschool sectors in hopes of improving the recruitment channels (Hagerman et al., 1994b).

The mode of service organization has an impact on both the recruitment channels leading to a diagnosis and on the management of affected individuals and their families. For better integration of diagnostic, medical management, reeducation and social integration services for fragile X individuals, at least two options seem possible to us. One, which can make do with the fragmentary structure of the services, would require a major informational and collaborative effort that could lead to the creation of a network that would be responsible for referring individuals whose needs are not in line with the mandate of a single organization in order to ensure them diagnostic and management services in a timely manner. This approach would be used not only for fragile X syndrome individuals, but for all individuals with multiple impairments. The other option would be based on the creation of centres where health, reeducation and integration professionals could work in multisectorial teams to provide families the following services: a diagnosis, a cognitive and functional assessment, genetic counselling, the development of an individualized service plan, and lastly, coordination of the necessary services. The mandate of such centres would be based on the awareness of the progressive nature of the manifestations of mental retardation, and they would therefore manage children with developmental delay or even language acquisition delay. Although this option is more likely to bring about true service integration, it would mean greater reorganization and more compromises, which would probably require some services to be relocated and the existing organizations' mandates and responsibilities to be reassigned. Whichever option is chosen, implementing these approaches will very likely require investments during the reorganization per se. However, reallocating resources that are too often devoted to scattered consultations and unstructured diagnostic workups would probably cover a portion of the recurring operating costs.

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[159] In the recent literature, several authors examined the more appropriate diagnostic protocols and point out that, with the most thorough assessments, an etiologic diagnosis could be made in a far greater proportion of developmentally delayed or mentally retarded children, this diagnosis often leading to more accurate genetic counselling for the family (Curry et al., 1997; Majnemar and Shevell, 1995; Battaglia et al., 1999).

[160] For example, a committee of the American Academy of Neurology and the Child Neurology Society recently proposed some guidelines, which included an algorithm, for screening for and diagnosing autism. The guidelines is composed of several steps, including screening for developmental delay and identifying children at risk for autism, followed by the diagnosing of autism and an assessment of the needs of the patients and their families (Filipek et al., 2000).

Together with the conceivable efforts regarding practices and the organization of services, the dissemination of information is of the utmost importance if one wishes to solve the main problems that have been identified. Providing better information to the various professional sectors involved in the offer of services would improve the recruitment channels and diagnosis, making it easier to obtain and coordinate services. It would also foster intersectorial collaboration and the exchange of expertise in the area of management methods. Moreover, a broader knowledge of the syndrome and of the services among the population would make for more appropriate support from the community for the families concerned and for more effective communication within these families. Lastly, informing parents is an essential component not only of genetic counselling, but also of psychosocial support.

6.4 CONCLUSION

No proactive high-risk-population screening strategy or no low-risk-population screening strategy can be recommended at this time, since such strategies raise numerous ethical issues that need to be debated and since a number of scientific and technical problems must first be resolved. The methods and knowledge in this area are evolving at an extremely rapid pace, with the result that, in the future, the situation will have to be reassessed in light of the new developments.

Consequently, for the time being, we will have to count on the improvement in the current practice of diagnosing affected individuals and of screening for people at risk for transmitting the syndrome to their offspring. This could be considered at several levels: that of professional practice and that of the organization of services, together with a wider dissemination of information.

From the standpoint of professional practices, developing and disseminating a standardized workup for developmental delay and mental retardation would have the advantage of promoting better diagnostic accuracy, which would be of benefit to all the children concerned. From an organizational standpoint, the problem of intersectorial coordination is the most obvious. Any effort to make improvements should essentially be aimed at promoting the integration of diagnostic services, the cognitive and functional assessment and the offer of reeducation and integration services. Such an objective could, for example, be achieved either by setting up a referral network or by creating truly multidisciplinary centres dedicated to mental retardation, but also to precursor signs, such as developmental delay or language acquisition delay. Any steps in this direction will mean greater responsibility on the part of the professionals and organizations involved as well as substantial cooperation.

7. ETHICAL AND SOCIAL ASPECTS

The objective of molecular fragile X diagnosis and screening is to help the families concerned by promoting early, individualized management of the affected children in hopes of giving them a better chance to develop their abilities and to alleviate the parents' psychological and social burden by reassuring them of their parental competence and their reproductive options. Furthermore, the members of the identified at-risk families can obtain genetic counselling, and once the risk in the potential carriers is determined, they can either have children without anxiety or be informed of the disease's mode of transmission and plan their families with the aid of prenatal diagnosis, if they so desire.

To maximize these potential benefits, a molecular fragile X diagnostic and screening service should take the following into account: 1) the situations in which the tests are ordered; 2) the organization of the services relating thereto; and 3) the impact of identifying the syndrome and of using genetic tests on individuals, families and society. The discussion of the socioethical aspects below covers the prerequisites for a high-quality diagnostic and screening service to be provided in a manner that is in keeping with individual rights and freedoms and the values underlying Québec's health-care system. After presenting the ethical and social aspects of diagnosing and screening for the syndrome in affected families, the discussion sheds light on the responsibilities of the professionals involved and of the public authorities. Given the limited number of studies on the ethical and social aspects that specifically concern fragile X syndrome, some of the aspects discussed pertain more generally to diseases characterized by mental retardation.

7.1 SOCIOETHICAL ASPECTS OF IDENTIFYING THE SYNDROME

While the potential benefits of diagnosing and screening derive from the possibility of making choices with full knowledge of the facts, untoward consequences are always possible. It is up to the authorities involved in the offer of this service to identify these consequences in order to prevent or at least minimize them. A few empirical studies[161] permit a discussion of those aspects which warrant consideration.

7.1.1 The impact of identifying the syndrome

The ethical issues raised by the identification of the syndrome concern the actual diagnostic workup, the communicating of the results and the follow-up that is subsequently proposed. Also, there are specific problems linked to the fact that fragile X syndrome is characterized by mental retardation: the stigmatization associated with it and the problems associated with genetic counselling and the dissemination of information (Nuffield, 1998).

The announcement that a newborn has a serious genetic disease comes as a major shock to the parents, one which often affects the quality of their personal, professional and family life (Dallaire, 1984). Unlike Down's syndrome or a hereditary disease that can be detected at birth, fragile X syndrome is rarely diagnosed in newborns. A diagnosis is generally made following the parents' request for a consultation because of a delay observed in their child. A positive diagnosis can then be favourably received, but it can also cause considerable disruption if the parents are not prepared or do not accept the sequelae of the syndrome, such as mental retardation. When the diagnosis is announced late, it can be more painful for the parents to say goodbye to having a normal child and to readjust their expectations (Cronister, 1996). Parents with a mentally retarded child prefer to obtain confirmation of this as soon as possible (Watkins et al., 1989).

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[161] These studies, most of which are exploratory, may be characterized by selection bias, and their results apply more to cascade screening than to low-risk-population screening.

A survey conducted among 151 members of families followed at a specialized centre in Colorado (Roy et al., 1995) explored parents' perception of the psychosocial impact of a diagnosis of fragile X syndrome and the medical and social services received after it was made. In this survey, 91% of the respondents thought that the diagnosis had been useful to them. The main reason given was that it had provided a better understanding of the child's problems and of their cause, thus removing the guilt and shame related to the false perception of personal responsibility in the occurrence of the child's problems (Roy et al., 1995). For most, the diagnosis did not change or slightly improved access to medical and social services[162]. The negative consequences of the syndrome that were reported included an increase in the psychological burden in 30% of the cases and rejection by other family members in 34% of the cases.

The hereditary nature of this syndrome implies that other children in the family may be affected, even though the problems are not yet apparent. A study concerning the experiences of 245 parents who were members of the UK Fragile-X Society showed that the time from when parents notice a problem in their child to when the diagnosis is made has considerably diminished over the last decade[163] (Carmichael et al., 1999). However, the diagnostic confirmation process and the information and psychosocial support given before and when the diagnosis is announced and after it is made could still be improved[164]. The fact that some professionals are not properly or adequately informed about fragile X syndrome is the main problem mentioned. If the clinical genetic follow-up is done properly and information communicated effectively within the family, a diagnosis of this hereditary disease will enable the relatives to access medical genetic services. For the 153 families which, between 1980 and 1990, received a positive diagnosis elsewhere than at a centre specializing in genetics, 22% were not referred for genetic counselling. Of all the parents questioned (n = 245), 16% were not aware of their risk of recurrence, and 25% were not aware of the risk for the relatives. In nearly one third of the families questioned (76 out of 245), when the diagnosis was confirmed in the first affected child, the parents had already had at least one other affected child (Carmichael et al., 1999).

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[162] These authors state that the population surveyed was already well served and that the results could therefore have been different if they had questioned parents who were not receiving services at a referral centre (Roy et al., 1995). Other authors in the United States point out that it is the amount of time it takes to confirm developmental delay (rather than a diagnosis of fragile X syndrome) that deprives children of early intervention services, which are, in principle, accessible everywhere in that country (Bailey et al., 2000).

[163] For children born between 1980 and 1990, the waiting time between the start of the diagnostic search and confirmation of the diagnosis decreased from a mean of 8.3 years (range: 4 to 12.3 years) to a mean of 1.2 years (range: 2 months to 3 years) (Carmichael et al., 1999). In a smaller study, Bailey et al. (2000) showed that, while a diagnosis of developmental delay was made at 23 months and a diagnosis of the syndrome at 35 months on average, it took 44 and 54 months, respectively, for these diagnoses to be confirmed in 90% of the 41 pre-schoolage boys with a full mutation.

[164] Of the 147 parents who expressed their opinion about this, 30 (20.4%) said that the professionals they had met with were attentive and empathetic with regard to the problem observed in the child and had provided adequate support, while 33 (22%) said the opposite. Of the 75 parents who commented on the announcement of the diagnosis, 47 (62%) found that it had been communicated in a respectful manner and with the appropriate support (Carmichael et al., 1999).

The impact of a diagnosis of fragile X syndrome therefore varies according to the setting, the circumstances in which the test is ordered and the individual or individuals concerned. However, in general, the impact is neither entirely positive nor entirely negative, based on the experience of people directly concerned (Carmichael et al., 1999). Such a diagnosis can have a significant benefit by providing an understanding of the child's problems and obviating the need for multiple consultations with specialists (Roy et al., 1995).

Giving birth to a child with a significant impairment, having to ensure that the child gets the best care available and, moreover, knowing that one is a carrier of a potentially recurring hereditary disease can cause psychological turmoil and change family and social relationships. A study involving 28 women carriers who had given birth to an affected child (McConkie-Rosell, 1997) discusses several factors indicative of both the positive and negative aspects of knowing the genetic risk. First, learning that they were carriers of the syndrome had altered their perception of themselves, but this impact was positive for half of them. Most did not feel guilty about having transmitted the syndrome but had experienced feelings of anger and depression over the fact of having an affected child, feelings that diminished with time. More than 80% thought that prior knowledge of their carrier status would have enabled them to make different family planning choices, and more than 90% would have preferred to know their carrier status before seriously embarking on a couple relationship. Lastly, all of these women recognized the importance of providing this information to their relatives, and, in 75% of the cases, they thought that a family member should perform this duty (McConkie-Rosell et al., 1997).

7.1.2 Dissemination of information within the families

Disseminating information within a family is essential for ensuring access to genetic counselling and the success of cascade screening (McConkie-Rosell et al., 1995; van Rijn et al., 1997). The dissemination depends, among other things, on the quality of genetic counselling and of the communication between family members, but it can also depend on the intellectual capacities of the individuals concerned. The few studies on this show that most of the individuals questioned about the importance of disseminating information within the family are aware of their responsibility. Nonetheless, communicating this type of information in a timely manner is sometimes difficult, especially if those concerned are in shock, knowing that they are carriers of a hereditary disease characterized by mental retardation. Communication is often more difficult with more-distant relatives (McConkie-Rosell et al., 1995). Although confirmation of an accurate diagnosis and genetic counselling can, in certain circumstances, facilitate communication on the part of the couple or even the relatives by promoting a better understanding of the problem (McConkie-Rosell et al., 1997), the feelings of shame, guilt or denial that this announcement can cause can also affect the quality of communication between the members of affected families (Roy, 1995) and therefore the use of services (McConkie-Rosell et al., 1995). In any event, the relatives' participation in genetic counselling is not always a given. Each person has the right to decide whether or not to seek counselling or to be contacted again for a follow-up. Participation also depends on the extent to which the individuals feel the need for counselling. Some opportune situations, such as a pregnancy or a plan to have a child, can move decisions along. Given these facts, strategies have been developed to facilitate the dissemination of information to relatives and to ensure proper understanding and recall of the information provided during genetic counselling (McConkie-Rosell et al., 1995).

7.1.3 The impact of raising an affected individual

Raising a child with fragile X syndrome can be a major psychosocial and family burden (Cronister, 1996; Meryash, 1989; Roy et al., 1995). A study comparing the perceptions of 31 women at high risk for transmitting the syndrome (including 16 obligate carriers) and the perceptions of 63 women at low risk showed that the high-risk women anticipated fewer problems raising an affected child than the control group women. Furthermore, the problems identified by the high-risk women were different from those anticipated by the low-risk women (Meryash, 1989). For the obligate carriers, the biggest problems were the financial burden and the impact on the handicapped child's social life and education. Their perception of the burden actually decreases with time and as the mother adjusts to her child's special needs. The couple relationship, informing friends and relatives that the child has fragile X syndrome, and obtaining services did not seem to be major problems (Meryash, 1989). The rate of divorce attributed to problems posed by raising an affected child is about 18 to 19% (Meryash, 1989; Lauria et al., 1992).

7.1.4 Use of prenatal diagnosis and attitudes toward aborting an affected fetus

Reproductive decisions and especially the use of prenatal diagnosis also require adequate support and services. In general, the PND uptake rate for fragile X syndrome is relatively high (see Section 6.1.2), but few studies have documented the difficulties experienced by couples faced with prognostically ambiguous results.

The acceptability of selective abortion depends, in part, on the perception of the burden of raising an affected child (Meryash, 1989). Apart from religious beliefs and the level of education, the main factors shaping attitudes toward abortion of a fragile X fetus derive from knowledge of the syndrome. A study examining attitudes toward abortion conducted among 33 women at high risk for transmitting the syndrome (including 17 obligate carriers) who received genetic counselling suggested that the acceptability of abortion in the context of a positive diagnosis depended on the understanding of the syndrome's genetic, clinical and behavioural aspects, and on the experience of having an affected child (Meryash, 1992). The perception of the burden of raising an affected child on social life and the perception of the negative impact on the quality of family life were determining factors justifying the acceptability of abortion, much more so than the perception of the risk of transmission (Meryash, 1992).

7.1.5 Attitudes toward screening and communicating the status of carrier in children

The molecular test is not just a diagnostic tool, and it can be performed at any stage of life, which raises the problem of ordering it at the appropriate time. The existence of a timely medical benefit for the child and of a substantial psychosocial benefit for the adolescent who is able to decide for him or herself constitute the cornerstone of the position taken by professional associations on this matter (ASHG/ACMG, 1995; Working Party of the CGS, 1994). Although there is no curative treatment for fragile X syndrome, early diagnosis in symptomatic individuals is considered a medical benefit and confers other advantages, which have been described at length. However, premutation carrier screening in children and adolescents poses an ethical problem. The utility, the appropriate time and the main issues concerning premutation screening in children in affected families were recently explored in about 60 parents who attended a national conference on the syndrome (McConkie-Rosell et al., 1999). The purpose of the study was to understand parental motives and concerns regarding the determination of positive carrier status in their asymptomatic children and informing the children of their status. Of 61 parents questioned, 31 (58%) had already informed their child of the hereditary nature of the syndrome, and 37 (61%) had already agreed to have their children screened, despite concerns, in 81% of them, about the risk of discrimination associated with screening.

On the whole, the study suggests that parents perceive an important responsibility with regard to the determination of their children's genotypic status and to informing them of it at the right time. This sense of responsibility is greater in mothers. Most (65%) of the parents thought they had the right to decide when the right time was to have their children screened and to inform them of the results. This attitude was justified by the fact of giving the children the opportunity to know what their carrier status is before they become sexually active. This parental concern seemed rather rooted in the desire to gradually prepare the child for the risk of transmitting the syndrome and for managing his or her carrier status than in the sole need to know or to alleviate their own anxiety (McConkie-Rosell et al., 1999).

7.1.6 The problems of stigmatization and discrimination

The problems of stigmatization and discrimination are most often brought up in connection with the use of genetic diagnosis for nonmedical purposes (Billings, 1992; Feingold, 1999; Gostin, 1991). These problems can occur more acutely in screening programs, such as those carried out in schools for fragile X syndrome (Billings and Hubbard, 1994). The sources of stigmatization are numerous. They can be the school, the community or those in charge of the screening. For example, the manner in which children who exhibit signs are identified and treated in relation to the other students can constitute a form of stigmatization.

Depending on the teachers' and school officials' attitude, a positive diagnosis can either contribute to more effective planning of the services required by these students or justify a reduction in these services, if the diagnosis is considered as a predictive factor of the limits of the child's development potential (Billings and Hubbard, 1994). In this regard, Roy et al. (1995) observed that a significant number of families had encountered stigmatization problems or had negative experiences in connection with the syndrome. Of the 151 parents questioned, 16% reported a negative reaction on the part of a teacher, 17% reported that the diagnosis of the syndrome resulted in negative labelling, and 16% reported that early labelling of the child contributed to limiting his or her development (Roy et al., 1995).

It is not inconceivable that genetic tests will be used to determine access to certain services, such as immigration, disability benefits and insurance, and to jobs (Royal Commission on New Reproductive Technologies, 1993; Privacy Commissioner of Canada, 1992; Knoppers, 1991). In an empirical study involving 332 members of different support groups for people with hereditary diseases (Lapham et al., 1996), 22% of them said that they or a member of their family had been denied health insurance, and 83% of these families had been asked, by an insurance company, about the occurrence of genetic diseases. One fourth of the respondents also said that they or a member of their family had been refused life insurance, whereas, in general, in the U.S. population, only 3% of applicants are turned down. About 15% of the respondents reported that they or a member of their family had been questioned about genetic diseases by their employer. Lastly, 21% of the respondents with a genetic disease said that they had lost or were refused a job because of their disease, while 9% had experienced the same fate because they had an affected child (Lapham et al., 1996).

As regards, more specifically, fragile X families, the studies are contradictory. Two suggest that about 30% of affected families suffer discrimination on the part of insurers and employers (Dorn, 1994; Roy et al., 1995). Testimony concerning the discontinuation of family health insurance coverage because of fragile X syndrome was reported in detail in an issue of the newsletter of the Colorado-based National Fragile X Foundation's (Stephenson, 1993). This testimony also suggests that a number of American insurers refuse health insurance for the mentally retarded, even in the absence of other medical problems.

The extent of the discrimination problem actually depends on the definition used by each author of what constitutes discrimination[165]. Another study, this one involving 39 fragile X families (Wingrove et al., 1996), showed that most of the affected individuals had been refused life or health insurance when the application was submitted after the diagnosis had been made and that, at best, insurance companies limited coverage to aspects of health that were unrelated to the syndrome. Yet, based on the definition used by these authors[166], only two cases of discrimination were reported. Apparently, the actual practice of discrimination is far less significant than the concerns expressed, given that nearly two thirds of the respondents stated that they were moderately or even extremely worried about their health insurance coverage (Wingrove et al., 1996).

It is up to the legislator to take measures to protect people against the use of genetic tests and information for nonmedical or commercial purposes. Some countries have started to prohibit the use, for insurance purposes, of genetic tests and information currently in medical records[167]. Presently, the Canadian insurance industry can ask applicants to undergo genetic tests or, more easily, question them about any genetic tests that they may have had (Lemmens and Bahamin, 1998). In this regard, the study by Lapham et al. (1996) revealed that 40% of the families had been questioned by their insurance companies about genetic diseases. Insurers generally consider that an applicant who knows what his or her risk or condition is has the obligation to inform them of that risk or condition, as is the case with one's family history. If consent is given, Canadian insurers have an almost unlimited right of access to an applicant's medical records to check the information that he or she has provided (Lemmens and Bahamin, 1998).

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[165] Genetic discrimination can be defined narrowly or broadly. Roy et al. (1995) consider, without distinction, the discrimination, stigmatization and prejudice suffered by affected individuals and the members of their families because of real or perceived differences in their genetic make-up. Similarly, Lapham et al. (1996) use the term "discrimination" to refer to prejudicial acts, as perceived by the respondents, toward them and the members of their families. For Billings et al. (1992), there is no discrimination if an individual who is symptomatic or who obviously has a genetic disease is refused insurance. Thus, the studies by Lapham et al. (1996) and Roy et al. (1995) do not permit one to identify the differences in treatment between individuals with a genetic disease and those who are not affected or to discuss the validity of the reasons for discrimination.

[166] The definition used by these authors was inspired by Billings et al. (1992). Genetic discrimination results from the improper use of genetic information in actuarial calculations for insurance purposes. In the authors' eyes, refusal to insure an entire family because of an affected child does not constitute discrimination (Wingrove et al., 1996).

[167] Some countries have already established a legal framework concerning the use of genetic tests by insurers and employers (see Comité consultatif national d'éthique pour les sciences de la vie et de la santé, 1996). The problem of using genetic tests and information for insurance purposes in Canada, together with an analysis of the provincial and federal legislation and of the international positions, is discussed in detail by Lemmens and Bahamin (1998).

In short, the socioethical aspects of identifying fragile X syndrome underscore the need for clear guidelines for the diagnostic and screening services, this to ensure their potential benefits and minimize the prejudice that can accompany the institution of such services. Genetic professionalswho made a significant contribution to the emergence of the current standards in medical genetics (Wertz and Fletcher, 1989) and the health and social services professionals involved in the diagnostic workup and in providing functional reeducation and social integration services, are both in direct contact with families and have a responsibility in this regard. Secondly, the legislator and the public authorities should put in place the necessary conditions for guaranteeing the quality and accessibility of the services offered and protect the individuals from any prejudice. It goes without saying that these services should be dispensed in accordance with the basic ethical principles of respect for a person's dignity, autonomy and privacy, and with the values that are part of the health-care system, such as fairness (Ministère de la Santé et des Services sociaux, 1998). These principles are recognized in the codes of ethics of health professionals, and the law provides protection in this regard.

7.2 SOCIOETHICAL ASPECTS OF PROFESSIONAL INTERVENTIONS

As for other hereditary disorders, the quality of fragile X syndrome diagnostic and screening services depends on the test's efficacy, on the quality of laboratory services and on the provision of clinical services, which require a sufficient number of competent personnel (Holtzman and Watson, 1997).

Given the complexity of the information to be provided, the familial nature of the syndrome and the psychological and social impact of diagnostic and screening practices, it seems essential to reassert the need for appropriate guidelines for the provision of molecular tests. A genetic test should be performed within the context of a medical procedure, with the practice standards and the protection that this context provides (Knoppers, 1991). The purpose of incorporating a genetic test into a medical procedure is to ensure that it is ordered for a recognized indication[168], that its administration is voluntary and accompanied by sufficient information for making a free and informed choice, and that its results are interpreted by competent professionals. Its incorporation into a medical procedure may seem to be an unnecessary affirmation in the Québec context, but this approach is faced with the rapid development of genetic tests in the form of diagnostic kits[169] and with the increasing involvement of scientists and physicians in technology transfer and the marketing of genetic tests (Silverman, 1995).

In general, the marketing of genetic tests raises several problems. If a certain number of companies are taking the initiative to market them, will it be possible to assess the quality of the diagnostic kits before they become commercially available? If several laboratories, both public and private, express interest in using these tests, will they have the necessary expertise to interpret the results, and will all of the laboratories fall under an accreditation and quality control system? Lastly, if these tests are sold over the counter or on the Internet, will it be possible to ensure that the diagnostic indications are adhered to and that the individuals tested receive an accurate interpretation of the results and professional genetic counselling?

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[168] The benefits of genetic tests can extend beyond the clinical context per se and include family planning and individual health choices, with the result that the interpretation given to the notion of medical indication may restrict the use of certain genetic tests. However, a medical framework has the advantage of minimizing the administration of tests for personal wishes.

[169] The problem raised here is not due to the development of diagnostic kits for laboratory use, but rather the possibility of developing kits that are so simple to use that selling them directly to the public or practitioners is conceivable.

In the case of fragile X syndrome, based on the available information, no over-the-counter test is available[170]. However, in the United States, many private laboratories currently offer diagnostic services for the syndrome. If less suggestive clinical signs are used as diagnostic indications, the test for fragile X syndrome could be administered to a rather large population of children, which would definitely be of commercial interest.

The absence of regulations in this regard is creating pressure on the current organization of the provision of molecular tests. The observed trend toward the privatization of genetic diagnostic services and the over-the-counter availability, in the United States and Great Britain, of a few genetic tests (ACGT, 1997) lead us to wonder how Québec's universal health-care system plans to incorporate molecular tests while at the same time preserving the values that guide this system and the principles that underlie medical genetic services.

Because of the impact on the family, the complexity of the genetic information and the difficult decisions based on it, medical genetics has adopted practice standards (Wertz and Fletcher, 1989; Modell et al., 1993; Andrews et al., 1994; Caulfield et al., 1995), which should be adopted by the other medical specialties that will be increasingly involved in the use and communication of genetic information (Holtzman and Watson, 1997). Although most of the ethical principles that underlie these standards are an integral part of medical deontology, they may receive a specific interpretation in the context of genetics (WHO, 1998). For example, while we easily equate respect for the autonomy of a person with the obligation to obtain his or her consent, the fact that genetic information is often provided in the absence of treatment means that respect for autonomy also implies respect for the decision to know or not to know one's possible genetic fate. Family support must also be understood in broader terms than simply providing information on reproductive risks and options. The psychological and financial burden of raising an affected child should be taken into consideration during such intervention (Cronister-Silverman et al., 1992). Lastly, hereditary disorders not only concern the person who seeks consultation, but also his or her family and the future generations. This is one more imperative that must be taken into account when planning genetic services. One must be able to coordinate the storage of these data and their timely communication.

In Québec medical deontology, when a hereditary disease is identified in a family, it is the individual who has sought consultation, not the physician, who has the responsibility of contacting the relatives to inform them of the family situation and, in this case, that it would be in their interest to seek counselling to find out what their risk is. This is very important for two reasons: respect for privacy and the maintaining of confidentiality. The confidentiality of the diagnosis is ensured by the health professionals. This information is not always available to people in the educational and social services sectors, unless the parents authorize its disclosure. Disclosing the diagnosis may have a positive effect on obtaining particular services, but it can also result in stigmatization (Billings and Hubbard, 1994), which can constitute an obstacle to social and school integration.

The right to privacy and the right to confidentiality of medical data, which, in principle, impose secrecy vis-à-vis the other family members and third parties, bring one of the most important dilemmas for medical genetics (Wertz and

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[170] This problem could mainly concern genetic susceptibilities to common multifactorial diseases, even if the first over-the-counter test available in Great Britain was for the purpose of detecting cystic fibrosis carriers (Harper, 1997).

Fletcher, 1989; Wertz, 1995). The American Society of Human Genetics recently examined these issues, which are still being debated. In its opinion, the familial nature of genetic information and the possibility of preventing serious consequences to family members are reasons that can be provided, in exceptional, well-defined, circumstances, for authorizing a breach of confidentiality (ASHG, 1998).

Testing minors for the purposes of determining their carrier status should be limited to adolescents of child-bearing potential who are capable of making decisions for themselves, in accordance with the guidelines issued by two American professional associations (ASHG/ACMG, 1995). Identifying a premutation in adolescents of child-bearing potential opens up all the reproductive options, including that of preventing the transmission of the syndrome, if applicable. However, it is of the utmost importance that the professionals who receive such a request weigh the benefits and risks in light of what is in the adolescent's best interest. Under Québec law, persons aged 14 years and older can, on their own, agree to procedures required for their health and even to procedures that are not required for their health if these procedures do not involve a serious risk or serious and permanent effects. Genetic counselling before testing and an individualized follow-up suited to the adolescent's situation are absolutely necessary.

7.3 SOCIOETHICAL ASPECTS OF PLANNING AND ORGANIZING SERVICES

Instituting a diagnostic and screening service is also a concern of the public authorities. From a health-care standpoint, the public authorities' responsibilities extend to the offer of services considered necessary, the allocation of resources to the different services, the organization of clinical and laboratory services, and quality assurance (Andrews, 1994; Holtzman and Watson, 1997). This responsibility is aimed at putting in place the necessary conditions for dispensing the services, ensuring their continuity and providing a follow-up in accordance with recognized professional standards.

Currently, in Québec and Canada, clinical genetics laboratories (cytogenetic, biochemical and molecular) are not subject to accreditation or quality monitoring. However, they do take guidance from good practice standards issued by the American College of Medical Genetics (ACMG, 1993). An overall perspective of quality requires that the tests be incorporated into the continuum of necessary services and responsibilities of all the professionals concerned. The quality of the genetic service should be assessed in light of the diagnostic and screening objectives: promoting informed choices by at-risk couples and enabling parents and professionals to better plan the strategies for developing the affected child's potential and for his or her social integration. Since reducing the incidence of the syndrome is not part of the primary objective, it cannot serve as the main assessment criterion. The quality of medical and social services supported by a sufficient number of competent personnel is a prerequisite for effectively instituting diagnostic and screening services.

If testing is to be ordered as part of every workup for developmental delay, steps will need to be taken to prepare a sufficient number of health professionals capable of providing referrals to orderers and enough orderers who can perform the diagnosis, communicate it, do a follow-up and provide referrals to more specialized services, including genetic counselling. Services provided to fragile X children are not specific but are similar to those provided to children with learning delay, language delay or mental retardation. Although integration into natural settings and regular classes is recommended, there are other options (special classes, special schools) that can meet the needs of fragile X children. However, there are regional disparities in the organization of such services. Also, the educational and health sectors do not seem to be very well integrated with regard to their approaches and do not collaborate enough to coordinate their interventions.

The numerous options available and the numerous parties involved, the lack of communication between the health and educational sectors, the lack of availability for ensuring effective service coordination, and the numerous cuts to special services are forcing parents to put in time and resources to obtain access to services. In short, the current context of sharing responsibilities for organizing services has significant consequences for the recruitment channels leading to requests for diagnostic tests and on accessibility to services for children and families.

Fairness requires that the organization of services be equitable, especially with regard to accessibility on a regional basis. Equal access to the available services is based on a health-care system that provides testing and required services within an appropriate amount of time in response to the need. To ensure equal access, certain conditions must be in place: access to a high-quality test, regardless of the region or orderer; promoting the full exercise of professional judgment as to the appropriateness of ordering the test, in particular, standardizing the institutional requirements for access to it; providing to the various professionals adequate training for educating, referring and supporting people who express needs; and making the general public aware of the disease, its signs, the existence of the test and the available services.

Instituting or maintaining new services is often justified by a comparison of the anticipated benefits and the incurred costs. All the benefits, including the psychological and social ones, should be factorable into economic analyses performed from a societal perspective (Mooney and Lange, 1993). However, certain benefits are far less tangible and difficult to measure, which makes an economic analysis a complex task. Furthermore, the benefits concern only certain families, yet the costs fall on all of society. Given these difficulties, the societal perspective is often marginalized. With the mounting pressure to reduce health-care costs, the offer of such programs could fall prey to this single economic logic. From this perspective, the cost of instituting and/or maintaining[171] services would be compared with the costs avoided, i.e., those associated with the handicap.

In the case of fragile X syndrome, several authors have attempted to determine the cost figures for this type of economic analysis. The cost estimate of managing an affected individual was very approximate, since the cost figures specific to the syndrome could not be obtained and since the estimate was based on the cost associated with the management of individuals with mental retardation (Turner et al., 1996; Lauria et al., 1992). A brief calculation of the avoided costs was made for a cascade screening program following proactive screening for affected individuals (Turner, 1986) and for a program for screening pregnant women (Ryynänen et al., 1999). Lastly, several authors have used theoretical models to explore the costs incurred and avoided for different low-risk-population screening strategies (Palomaki, 1994; Meadows and Sherman, 1996; Wildhagen et al., 1998; Vintzileos et al., 1999). Their estimates are relatively discordant and are very sensitive to the assumptions concerning the test costs and the prevalence of the mutations, among other things. Wildhagen et al. (1998) conclude that there are no economic obstacles to using molecular tests for these types of screening[172] and that the decision to set up a screening program should be based mainly on a discussion of the medical, psychological, social and ethical issues associated with this practice. Indeed, an economic analysis is insufficient for assessing the usefulness of instituting the service, especially because of its limitations and particularly, in the field of genetics, because of its assumptions concerning the objectives of screening.

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[171] Every laboratory service involves recurring costs, which depend on the average cost of the tests, the available equipment and the number of tests performed. Genetic counselling services result in additional costs.

[172] Despite the differences in the methods and in the cost figures used, the conclusions of the other authors are simi-

In medical genetics, economic analyses raise numerous concerns (President's Commission, 1983). The objectivity sought in economic analyses does not lend itself easily to the particularities of genetic services aimed at providing information to individuals and families so that they can make informed reproductive or preventive choices (NERGG, 1997). Although this economic evaluation may be useful in the policy development process, serious ethical questions arise when the analyses are essentially aimed at the avoidance of the costs associated with aborting affected fetuses (Moatti et al., 1992). This type of analysis does not, therefore, take into account the value attached to life (whether it is a life avoided or a life in good health owing to prenatal diagnosis), the quality of life of handicapped individuals or couples' preferences (Shackley and Cairns, 1996; Ganiats, 1996; Andrews et al., 1994). More and more, in genetics, the utility of a screening service is assessed in light of the benefits for the families concerned (meeting a need, improving the quality of life, not doing any harm) and, to a lesser degree, for the general population (reducing the direct and indirect costs of the management of individuals with mental retardation by optimizing expenses) (Marteau and Anionwu, 1996). In any event, although it is true that these services reduce the number of affected individuals born and the costs associated with the handicap, this effect will always depend on the couples' choices and preferences.

An approach aimed at calculating the costs per identified carrier avoids, in part, these pitfalls (Modell et al., 1993), for in recognizing that detection of carriers, not only affected fetuses, is a valid benefit, this approach attaches importance to the information provided to the families, the benefits they derive from it as regards their reproductive choices, the personal choices of couples and the option available to them of having healthy children. Even this type of economic analysis is complex, and it is beyond the scope of this report. Without wishing to diminish the importance of economic factors, it seems appropriate to emphasize the fact that the objectives sought are not primarily based on economic considerations.

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lar, except those of Vintzileos et al. (1999), who arrive at the opposite conclusion for prenatal screening. Their analysis, which is briefer, is based on a higher test cost (consistent with the use of the Southern method instead of PCR) and a much lower estimate of the management costs.

8. CONCLUSION

Fragile X syndrome is the most frequent cause of hereditary mental retardation and the second leading cause of mental retardation, after Down's syndrome. Fragile X syndrome has been identified in more than one third of families in which X-linked mental retardation is found. Mental retardation is the most prominent characteristic of the syndrome, but its manifestations vary from individual to individual and appear gradually with age. The earlier signs, such as language acquisition delay, are often the least suggestive. Subsequently, behavioural problems and dysmorphic signs appear as well. Since no sign is pathognomonic and since the most typical signs (facial dysmorphia and macroorchidism) appear late, early clinical diagnosis is difficult.

This monogenetic disease exhibits an unusual mode of transmission. It is X-linked but dominant, with variable penetrance. It affects both sexes, but boys are generally affected more severely than girls. Approximally 55% of affected girls develop mental retardation, usually mild to moderate, while mental retardation is moderate or more severe in more than 90% of affected boys. Both males and females can be asymptomatic carriers of the mutated gene and are therefore at risk for transmitting the syndrome to their offspring.

The identification of the FMR1 gene in 1991 and the characterization of the type of mutation associated with it constitute a major advance in the understanding of the hereditary transmission of the syndrome. The mutation in question is a dynamic mutation, so called because it involves an unstable DNA segment that can undergo expansion during maternal hereditary transmission. This phenomenon explains transmission of the syndrome by asymptomatic males and females (premutation carriers) and its unexpected occurrence in individuals in the absence of a family history of mental retardation (with increased probability of clinical expression in subsequent generations).

Molecular analysis of the FMR1 gene, which has been available as a clinical service since 1992, constitutes a substantial gain in relation to the previous cytogenetic analyses, which were prone to classification errors and relatively unreliable in females. The molecular test clearly establishes a diagnosis in symptomatic individuals, detects individuals at risk for transmitting the syndrome and, by identifying the type of mutation, permits a more accurate estimate of the risk of transmission of the syndrome in an affected individual's relatives. These developments have contributed significantly to the genetic counselling of affected families, reassuring some and permitting the others to make their reproductive decisions with full knowledge of the facts.

Genotypic analysis for fragile X syndrome is based on the use of two techniques, Southern blot and PCR, which are used sequentially. There are two laboratories in Quebec that offer molecular tests for the FMR1 gene. While one of them has been using the reference method since 1991, the other developed more recently an alternative method, the analytical validation of which has raised certain questions. This situation, in which a nonvalidated test is being used, is not unique in genetics and is part of a process of transferring research results to clinical services that is not governed by any independent form of evaluation or system of approval. However, it would not be desirable for similar situations to occur or for there to be more than two laboratories in all of Québec offering molecular testing for this syndrome for the time being.

It would be beneficial if the validation done collaboratively by the two laboratories were to result in the harmonization of the diagnostic protocols. In addition, quality control should be performed periodically, as should be the case for all other molecular tests. Moreover, closer collaboration between genetic services and laboratories would make for a closer follow-up of the diagnoses (prenatal and others), thus contributing both to monitoring the clinical and laboratory activities and advancing epidemiological and molecular knowledge.

The birth prevalence of the syndrome is estimated to be at least 1 per 4,000 males and about 1 per 8,000 females. In Québec, as in most countries, the syndrome is underdiagnosed. When the diagnosis is made, it is still made rather late, with the result that the nuclear families cannot always benefit from genetic counselling in a timely manner. Also, most known families have not been evaluated completely, since the relatives of the affected individual do not always feel the need to seek out the available services for the time being and since the stigma attached to mental retardation can affect the sharing of information within the families.

Because of the complexity of this hereditary disease and of the genetic tests, several conditions must be in place in order to judiciously incorporate them into medical practice. The offer of testing should be accompanied by genetic counselling tailored to the specific characteristics of the syndrome, in order to provide complete and adequate information and the necessary psychosocial support when providing this information. Genetic counselling is all the more tricky because our current knowledge regarding several aspects of the pathological mechanisms of the mutations is still lacking, which has an impact on making a prognosis and on reproductive choices.

If there are clear guidelines for the offer of molecular testing, its contribution will be substantial with regard to the accuracy of the diagnosis and identifying individuals at risk for transmitting the syndrome to their offspring. Furthermore, these tests are paving the way to various diagnostic and screening strategies. Several arguments can be put forth for the appropriateness of low-risk-population screening: the syndrome is underdiagnosed; about one third of the cases of the syndrome occur in families with no family history of mental retardation; the prevalence of premutations is high; and the proportion of carriers who can be identified through high-risk-population screening followed by cascade screening is reportedly much lower than the results anticipated for low-risk-population screening (Wildhagen et al., 1999).

While strategies for identifying premutation carriers are essentially aimed at genetic counselling, those aimed at better identification of the syndrome in children with suggestive signs can, in principle, confer a double benefit to families by way of genetic counselling and an improvement in the management of their affected children. The assessment of the different proposable diagnostic and screening strategies is based on utility, acceptability and feasibility criteria and takes into account international experience and an assessment of the situation in Québec. To a large extent, the situation in Québec underlies the considerations regarding the feasibility of the various screening strategies but also colours the issues of utility and acceptability. For example, to determine if, apart from the benefits associated with genetic counselling, making an earlier diagnosis confers any benefits to the affected individual and his or her family, one must take into consideration the services that are presently available to them.

An examination of the situation in Québec reveals deficiencies in the recruitment channels leading to a diagnosis and in the follow-up and management of affected individuals and their families. The variability and insidious onset of the symptoms, the relative lack of knowledge of this clinical entity by first-line health professionals, the numerous points of entry into the system, the lack of collaboration between the health-care system and the school and preschool system, and the absence of a standardized workup for developmental delay and mental retardation lead to highly variable recruitment channels and unplanned prescreening, and result in fragile X syndrome being underdiagnosed. Furthermore, the "prediagnostic" path is described by parents as being difficult and painful, and the psychosocial support after the diagnosis is announced, timely access to genetic counselling, and referrals to the necessary support and social integration services vary considerably.

As for follow-up and management, many organizations and professionals have responsibilities toward individuals with developmental delay, mental retardation and/or behavioural problems. The families concerned could therefore, in principle, avail themselves of various management modalities both in the medical, social and educational sectors. In practice, however, the diversity and scatter of the resources undermine their optimal utilization and generate problems of accessibility to services, thus compromising their continuity and complementarity. Despite the efforts that have been made in the health and social services system to improve coordination from an organizational standpoint, and despite the potential that developing individualized service plans has for fostering service continuity and complementarity at the individual level, it must be recognized that the lack of communication and collaboration between the medical, social and educational sectors continues to hinder access to services. Furthermore, the availability of certain services has been compromised by past years' budget cuts and the shortage of certain human resources in the health and social services and educational systems. These circumstances place a heavy burden on the families and can have negative consequences on the children's development.

With respect to the objective of screening for asymptomatic carriers at risk for transmitting the syndrome to their offspring, it seems that a larger proportion of premutation carriers can be identified by strategies that directly target low-risk populations than those that involve screening for affected individuals followed by cascade screening. However, because of technical reasons, the feasibility of these approaches is a problem (availability and validation of tests suitable for wide-scale use), and their acceptability is hindered by ethical problems due to the limits of our scientific knowledge (predicting the prognosis).

As for the objective of improving the diagnosis and management of affected individuals, proactive high-risk-population screening strategies could, based on international experience, prove effective, but their feasibility in Québec is compromised due to organizational reasons (no concentration of at-risk individuals either in the health and social services system or in the educational system), and they have not achieved acceptability because, among other things, the public and professionals are ill-informed about the syndrome.

Our assessment therefore leads us to conclude that no proactive high-risk-population screening strategy or no low-risk-population screening strategy can be recommended at this time due to the facts that such strategies raise numerous ethical issues which need to be debated and that a number of scientific and technical problems first need to be resolved. The methods and knowledge in this area are evolving at an extremely rapid pace, with the result that, in the future, the situation will have to be reassessed in light of the new developments. For the time being, we will therefore have to rely more heavily on an improvement in the current practice of diagnosing affected individuals and persons at risk for transmitting the syndrome to their offspring.

Other considerations argue in favour of this option. First, service continuity is one of the objectives of the Québec health-care system and should be implemented both at the service organization and service distribution levels (*An Act respecting health services and social services*) and at the level of medical practice (obligation to provide follow-up). Second, the responsibilities involved in instituting a diagnostic and screening strategy cannot be seen to be limited to offering the test and to medical and genetic management. They should include, more broadly, the services required by affected individuals and their families. In such circumstances, it seems logical to meet the needs of symptomatic individuals first so as to ensure that one can adequately meet the needs of anyone who might eventually be identified in the event that all the conditions are in place for proposing other diagnostic or screening strategies.

Given the deficiencies observed in the offer of services, improving the current practice would require better access to earlier diagnosis and better accessibility, continuity and complementarity with regard to the medical, social and educational services provided to the families concerned. While there may not be any recognized direct therapeutic benefit for affected individuals, early diagnosis would provide appreciable benefits in terms of defining their needs and those of their families. Confirming the diagnosis in a symptomatic individual could thus contribute to better planning of the necessary care and services, even if this contribution is difficult to assess[173]. Maximizing the benefits of early diagnosis therefore requires efforts to improve the referral of affected individuals and their families to the available services[174], to improve to the available services[174], to improve timely access to the necessary services[175] and to adapt the available services to the specific needs of affected individuals and their families. This objective cannot be achieved unless a substantial effort to provide information to professionals enables them to use the diagnosis as a service planning and coordination tool.

As regards accessibility to services, efforts should be considered at several levels: that of professional practices and that of the organization of services, together with broader dissemination of information. As for professional practices, developing and disseminating a standardized workup for developmental delay and mental retardation would have the advantage of improving the diagnostic workup, which would be of benefit to all the children concerned. The main problems encountered result from a lack of intersectorial collaboration. This state of affairs is also due, in part, to the professional practices (conceptual differences and lack of communication), but mainly to organizational (the organizations' missions and responsibilities) and circumstantial (lack of information, lack of human resources and budget cuts) aspects. The compartmentalization of services between the medical, social and educational (and job) sectors hinders case-finding and the adequate management of affected families. Better integration of diagnostic, medical management, reeducation and social integration services should therefore be sought, both for fragile X individuals and those with mental retardation, developmental delay or other developmental problems of known or unknown etiology.

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[173] Demonstrating the usefulness of the diagnosis with respect to adapting the educational and functional reeducation interventions, for example, would require research specifically concerning affected individuals, research for which more systematic diagnosis would, in fact, be a prerequisite.

[174] In this connection, it will be recalled that more adequate relaying between medical services and reeducation and social integration services is very much desired and that parents need information and guidance in order to obtain services.

[175] In particular, we are thinking of the importance of early intervention on the part of functional reeducation professionals, especially in the presence of speech problems; of the quick referral of families to genetic services so that they can receive genetic counselling and undergo cascade screening, if they so desire; and of the importance of psychosocial support for families, especially when the children have behavioural problems in addition to the mental retardation.

The problems raised with regard to the offer of services to fragile X individuals are, on the whole, those that concern all mentally retarded individuals. Our observations concur with the Ministère de la Santé et des Services sociaux's description of the system's strengths and weaknesses (MSSS, 1999) in its consultation document drafted for the purpose of revising the social integration policy. In that document, it is pointed out that the often late identification of mental retardation limits timely access to services and that the lack of human and financial resources limits the availability of certain services and hinders access to services. However, emphasis is placed mainly on the need to create a network of truly integrated services, which would require cooperation at the local, regional and provincial levels. The current lack of intersectorial collaboration is attributed, among other things, to the lack of training of the professionals in the various sectors with regard to mental retardation and "to some confusion over the roles and responsibilities of the different organizations concerned" (MSSS, 1999). In this regard, however, fragile X individuals are more likely than others to be the victims of the organizations' policies of exclusion and to be bounced from one service to another (limiting service access and continuity) because of the complexity of their clinical picture[176] and the relative lack of knowledge of the syndrome.

Apart from the steps taken at the ministerial level to promote the social integration of the mentally impaired, it seems that substantial efforts should also be made at the regional level by way of cooperation involving, among others, the regional boards, the child care services, the school boards and the job sector in order to determine the division of responsibilities and to make the most of the available expertise.

Consequently, the Agency believes that:

1. The necessary medical, social and educational resources should be available to meet the needs of fragile X families in a timely and appropriate fashion.

2. The different players in the health and social services and educational systems should examine possible ways of improving early identification and the diagnostic workup of children with signs consistent with fragile X syndrome, devoting special attention to the services available for developmentally delayed children.

3. One or two laboratories should be available to perform, for all of Québec, the molecular tests for fragile X syndrome in the following situations:

    - The molecular diagnosis of fragile X syndrome in a symptomatic individual with either an indication recognized in the medical association guidelines or signs consistent or associated with the syndrome, in the opinion of the ordering physician.

    - Cascade screening of an affected individual's relatives.

    - Confirmation of carrier status in a pregnant woman with a family history of signs associated with the syndrome.

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[176] Although most of the needs of affected individuals are due to mental retardation, language acquisition problems, learning disabilities, behavioural problems and socialization problems give rise to other needs, which must be taken into consideration. Unfortunately, the coexistence of several types of problems results in the fact that the clinical picture does not fit perfectly with the mission of the various organizations involved in the offer of services.

- Prenatal diagnosis, if the mother is a carrier of a premutation or a full mutation.

4. All laboratory services should be subjected to quality control.

5. The different players in the health and social services system, the educational system and the job sector should improve intersectorial collaboration at the regional level in order to improve the coordination and continuity of the services available to affected individuals and their families.

6. The issues of the accessibility, continuity and complementarity of services for fragile X syndrome reflect, in part, an organizational problem that also affects children with developmental delays of other etiologies and their families, with the result that the required efforts should be part of a coherent approach that will benefit all these families.

7. The public authorities should give preference to a mode of service organization that promotes the respect of individuals, ensures equal access to services in all regions and prevents discrimination, especially in the area of insurance.

8. Research should continue, here and elsewhere, to better document the following:

    - The epidemiology of the syndrome in the general population.
    - The risk of hereditary transmission of the syndrome.
    - Phenotype prediction.
    - The development of genetic tests better suited to wide-scale use.
    - The psychosocial impact of diagnosing, screening and genetic counselling.

9. It would be essential to evaluate, by means of pilot projects, any high- or low-risk-population diagnostic and screening strategy whose implementation might be considered, on the basis of the following criteria:

    - Its technical, organizational and economic feasibility.
    - Its efficacy in terms of the number of individuals or couples who have received genetic counselling and a follow-up that meet their needs.
    - Its utility in terms of the services that are already available.
    - Its ethical and social acceptability.

APPENDIX I:

EVOLUTION AND CURRENT STATE OF KNOWLEDGE DERIVED FROM GENETICS

APPENDIX I: EVOLUTION AND CURRENT STATE OF KNOWLEDGE DERIVED FROM GENETICS

In this appendix, we summarize a few highlights of the evolution of the knowledge[177] that led to the identification of the FMR1 gene. These discoveries were based on a reverse genetics approach using both genetic and physical mapping.

Localization of the gene

Thanks to the efforts of numerous researchers, the close link between the FRAXA site, which is located at Xq27.3, and the gene associated with the syndrome was discovered by linkage analysis and *in situ* hybridization.

Localization of the CpG island

The existence, near the fragile site, of a CpG island whose methylation correlates with the syndrome's phenotypic expression was discovered by Vincent et al. (1991) and Bell et al. (1991). These CpG islands, which are linked to the 5' end of many genes, consist of sequences rich in cytosine-phosphate-guanine dinucleotides and are probably involved in the regulation of the transcription of these genes. The methylation status of the island's cytosine residues is determined by the effect of restriction enzymes that only cleave their restriction sites in the absence of methylation. These residues are usually methylated on inactive genes and unmethylated on transcribed genes.

Identification of an unstable DNA fragment

Different methods have been used in order to isolate DNA fragments corresponding to sequences near the CpG island (Heitz et al., 1991). The physical map of the region will gradually be refined, using these fragments as probes and different restriction enzymes. Several teams have, in this way, identified a DNA fragment of variable electrophoretic mobility and therefore of variable length (Oberlé et al., 1991; Kremer et al., 1991; Yu et al., 1991; Verkerk et al., 1991).

This allelic instability seems to be correlated, to a certain degree, with the individual's pedigree position, the syndrome's expression and the methylation status of the CpG island. Oberlé et al. (1991) propose that alleles containing between 150 and 500 base pairs more than normal alleles be considered premutations and larger alleles full mutations. Premutations are observed in normal transmitting males and their daughters and in female carriers with a negative or borderline cytogenetic analysis, while full mutations are observed in affected males and in females expressing the fragile site. Premutations are not methylated on the active X chromosome, while full mutations are. However, it is immediately apparent that these correlations must be qualified because of the existence of allelic and methylation mosaics (Rousseau et al., 1991a).

Identification of trinucleotide repeats

The allelic instability was attributed to the existence of a sequence containing a variable number of trinucleotide repeats (Kremer et al., 1991; Verkerk et al., 1991; Fu et al., 1991). The sequencing of alleles from normal and affected individuals confirmed that the only difference in terms of nucleotide sequences is the number of CGG (cytosine-guanine-guanine) trinucleotides (Fu et al., 1991) and that this mechanism constitutes the very nature of the mutations involved in fragile X syndrome. These mutations were subsequently called "dynamic mutations".

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[177] For further details, see Pellissier et al. (1993), Nussbaum et Ledbetter (1995), Brown (1996), Eichler et Nelson (1998) and Kaufmann et al. (1999).

The discovery, on the same small DNA fragment, of the CpG island, the trinucleotide repeat and breakpoints corresponding to the fragile site led to the hypothesis that the trinucleotide repeat also coincides with the FRAXA site. This seems to have been confirmed by *in situ* hybridization of these probes on both sides of the fragile site and at the site per se (Verkerk et al., 1991; Fu et al., 1991).

Identification of the FMR1 gene

To identify the gene involved in fragile X syndrome, the correspondence between the probes covering the CpG island region and a library of DNA complementary to messenger RNAs isolated from brain was studied. In so doing, Verkerk et al. (1991) identified a gene, FMR1, situated near the CpG island and whose 5' end contains the trinucleotide repeat. The description of the gene was completed by Eichler et al. (1993). The expansion is located in the nontranslated portion of the first exon.

Transcription of the FMR1 gene into mRNA is reduced or absent in affected individuals, and this expression of the gene seems to be correlated with methylation of the CpG island (Pieretti et al., 1991; Sutcliffe et al., 1992). Fragile X mental retardation protein (FMRP) is not detectable in individuals with a totally methylated full mutation but is detected in premutation carriers (Devys et al., 1993; Verheij et al., 1993).

APPENDIX II:

TECHNICAL ASPECTS

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This appendix provides a more detailed discussion of the technical aspects of the main tests used to diagnose affected individuals and to screen for asymptomatic carriers of fragile X syndrome: cytogenetic analysis, the Southern method, PCR and immunocytochemical analysis of FMRP[178].

II.1 CYTOGENETICS

Before molecular tests were developed, fragile X syndrome was diagnosed by a specific cytogenetic method. Although this technique was widely used to diagnose symptomatic males, it was very quickly realized that it did not perform well when it came to identifying asymptomatic carriers, since normal transmitting males and more than 50% of females who are obligate carriers do not express the fragile site (Sherman et al., 1984).

This cytogenetic approach was developed in the wake of Sutherland's observations (1977, 1979) that hereditary fragile sites[179] could be detected in a laboratory by placing lymphocytes in appropriate cell culture media prior to chromosome marking. The diagnosis was therefore based on identifying the FRAXA fragile site, which is on the long arm of the X chromosome (at Xq27.3). The culture media that induce expression of the FRAXA fragile site are those that interfere with the synthesis of the pyrimidine bases required for DNA replication[180]. A similar culture-and-analysis technique was used as early as 1981 on fetal cells obtained by amniocentesis, chorionic villus biopsy or cordocentesis for prenatal diagnosis (Jenkins et al., 1981).

Unlike the other chromosomal abnormalities (detected by standard cytogenetic analysis or karyotyping), which are usually present in all the cells of an affected individual, the expression of the FRAXA fragile site does not occur in all the cells examined from an affected individual. The frequency of cells expressing the fragile X site varies considerably from one affected individual to another, ranging from 1 to 50% (Dewald et al., 1992), but it also depends, to a certain extent, on the induction methods used, other experimental conditions (pH, duration of culture) and the quantity of folic acid consumed by the individual.

Given the array of techniques used and their effects on the results, several guidelines were issued by professional organizations between 1989 and 1991 for fragile X cytogenetic analysis using peripheral blood lymphocytes. The guidelines were mainly established by consensus and drafted by committees consisting mainly of cytogeneticists. A summary of the recommendations was prepared during the IVth International Workshop on the Fragile X Syndrome and X-Linked Mental Retardation (Jacky et al., 1991).

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[178] We shall not, in this report, discuss linkage analysis, which was used for a brief period before the development of direct molecular tests.

[179] A fragile site is a specific region on a chromosome where the chromatin is not compacted enough. Thus, part of the chromosome seems to be attached to the rest by only a thread. Cytogenetically, this phenomenon appears either as a vacuole unstained by the usual marking methods between two chromosome portions or, more rarely, as a complete break in the chromosome. Currently, six fragile sites have been identified on the X chromosome: FRAXA (FMR1), FRAXB, FRAXC, FRAXD, FRAXE and FRAXF. In the region next to the FRAXA site, there is the common fragile site FRAXD at Xq27.2 and the rare fragile sites FRAXE and FRAXF at Xq28. Only the FRAXA and FRAXE sites have been associated with a clinical phenotype, with mental retardation in both cases (Knight et al., 1993; Mulley et al., 1995).

[180] These induction methods involve thymidine or folic acid depletion of the medium (Sutherland, 1979), the addition of thymidylate synthetase inhibitors (methotrexate, trimethoprim, 5-fluorodesoxyuridine (Glover, 1981) or metabolic blockade due to excess thymidine (Sutherland et al., 1985).

These various guidelines were evaluated (Dewald et al., 1992). The recommendations were basically aimed at establishing practice standards for 1) fragile site induction methods[181]; 2) diagnostic criteria and results interpretation; and 3) quality control[182].

As regards diagnostic criteria, it was mainly a question of determining the minimum number of cells to be examined and the minimum proportion of cells in which the fragile site had to be found in order for one to arrive at a positive result. While the figure previously used for this purpose could vary from 2 to 4% (sometimes even 1%), the minimum proportion set out in the guidelines is 4%[183]. The reason for this is that a background of fragile sites in 1 to 2% of cells can be detected in individuals who are not fragile X carriers. Proportions of 3 and 4% should be considered questionable results requiring further investigation (Jacky et al., 1996). There is no general agreement as to the number of cells that should be examined[184]. While some expert groups recommend examining 100 cells in males and 150 in females (Jacky et al. 1991; Jacky, 1996), Dewald et al. (1992) believe that examining 50 or 75 cells, respectively, is enough, provided one examines more cells if a low positivity rate is obtained for the original sample.

As for interpreting the results, when a fragile site is identified on the X chromosome, it should be confirmed that this site is indeed the FRAXA site at Xq27.3. Thanks to chromosome marking, the FRAXA site can be distinguished from the FRAXD site, located at Xq27.2 (Sutherland and Baker, 1990). However, cytogenetic analysis is unable to differentiate the FRAXA site from the FRAXE (Knight et al., 1993) or FRAXF (Ritchie et al., 1994) site, which can be a source of false-positive results.

Despite these developments, which occurred when molecular analysis was about to supercede the cytogenetic approach, a number of problems persist with regard to quality control for the preparation of samples for detecting the fragile site, the process of detecting the fragile site and the diagnostic criteria. All of these aspects affect the validity of the cytogenetic test. Discrepancies between the results of cytogenetic analyses and molecular analyses have been demonstrated both in the diagnosis of affected individuals and in prenatal diagnosis.

For example, a British team sought to validate, by molecular analysis, the cytogenetic diagnoses made during a fragile X screening project among children with learning disabilities or mental retardation (Webb et al., 1986; Morton et al., 1997). Of the 16 boys and 13 girls diagnosed in the original study, only five of the 14 boys and two of the ten girls who agreed to be retested were found to carry a full mutation of the FMR1 gene. Two boys and two girls actually expressed the common site FRAXD, while none of the children identified in the original study expressed the FRAXE or FRAXF site (Morton et al., 1997).

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[181] To obtain optimal cell culture and chromosome preparation conditions (Jacky, 1991), expression of the fragile X site requires a combination of at least two systems of induction (by depletion and by antagonism), which requires two cell cultures.

[182] Quality control basically involves checking the effectiveness of the induction system. Two approaches are possible: including a positive control (sample from an affected individual), which makes the procedure more cumbersome (Jacky et al., 1991), or identifying fragile sites on the other chromosomes (Jenkins et al., 1990), which is not always reliable (Dewald et al., 1992).

[183] Of 1,033 cases referred for fragile X cytogenetic analysis between 1985 and 1990, 38 positive cases were identified (33 males and 5 females). However, four cases involving females were not considered positive because the frequency of fragile X cells was 1% in three cases and 3% in one case (Dewald et al., 1992).

[184] See Dewald et al. (1992) for a discussion of the various guidelines.

As for prenatal diagnosis, a retrospective study showed that of 143 positive cytogenetic diagnoses performed at 17 laboratories between 1981 and 1992, two cases turned out to be false positives and, at the time of the study, 18 false-negative cases had been identified (Jenkins et al., 1995). The false-negative cases could be explained by a positive-cell frequency of less than 4%. The false-positive cases can be explained by the presence of other fragile sites on the X chromosome.

As regards evaluating fragile X families, a multicentre study involving 318 families showed that a low or positive fragile site frequency (limit varies from centre to centre) was found upon cytogenetic analysis in only 12% of the males and 21% of the females with a premutation (Rousseau et al., 1994). Furthermore, in the full-mutation and mosaic carriers, no fragile sites were identified in about 1.5% of males and 13% of the females, and a clearly positive result was obtained in only 94% of the males and 74% of the females.

The main limitation of the cytogenetic method is, therefore, that it does not permit identification of all females carriers or of normal transmitting males, since it does not reveal most premutations or detect all full mutations, especially in females. As a result, the syndrome cannot be ruled out if a negative result is obtained. **Consequently, screening for individuals at risk for transmitting the syndrome in affected families is not feasible, which considerably limits the possibility of genetic counselling.**

Furthermore, the entire process, from culturing to obtaining results, including karyotyping, is especially long and labour-intensive. It was estimated that an experienced technician could perform about five analyses a week (Dewald et al., 1992; Jenkins et al., 1992), which makes this an extremely expensive test.

From 1979 to 1991, confirmation of fragile X syndrome was based on the identification of a fragile site at Xq27.3. Subsequently, however, general agreement quickly emerged over discontinuing cytogenetic diagnosis, with molecular diagnosis supplanting the cytogenetic diagnosis of fragile X syndrome, both for prenatal diagnosis and for confirming a diagnosis in an affected individual (Oostra et al., 1993; Jenkins et al., 1995).

In cases of mental retardation or developmental delay and in the absence of a known family history of the syndrome, karyotyping (usual cytogenetic analysis, which is nonspecific for fragile X syndrome) is, however, indicated for ruling out other chromosomal abnormalities, since many laboratories that perform tests to confirm fragile X syndrome have observed other such abnormalities at frequencies of between 2 and 5% (Hagerman et al., 1988; Voullaire et al., 1989; Dewald et al., 1992; Jenkins et al., 1992; Turner et al., 1992; van den Ouweland et al., 1994; Howard-Peebles et al., 1995; Marini et al., 1997). Studies involving a systematic assessment of children with mental retardation of unknown etiology report chromosomal abnormality rates of about 10 to 12% (Majnemar and Shevell, 1995; Battaglia et al., 1999).

II.2 SOUTHERN METHOD

The Southern method involves cell membrane digestion, isolating the DNA, digesting the genomic DNA with restriction enzymes, separating the DNA fragments by electrophoresis, transferring the DNA onto membranes and hybridization with radioactive complementary DNA (cDNA) probes in order to visualize and identify the DNA segments of interest.

Several restriction enzymes and probes that were utilized to explore the region near the CpG island and to reveal the unstable DNA fragment were subsequently used for diagnostic purposes. The first molecular analyses of affected families were performed in this manner by Oberlé et al. (1991), Rousseau et al. (1991a), Hirst et al. (1991), Yu et al. (1991, 1992), Verkerk et al. (1992) and van Oost et al. (1992), and the first prenatal diagnosis by direct detection of the mutation was performed by Sutherland et al. (1991).

Different authors have used protocols that differ both in the choice of restriction enzymes and in the choice of probes[185]. The advantages and limitations of different methods and the precautions to be taken when performing and interpreting the tests are discussed by Rousseau et al. (1992) and Snow et al. (1993).

Among the restriction enzymes, there are those whose restriction site is in the CpG island and whose action is sensitive to the island's methylation (EagI, SacII, BssHII and BstZI) and those whose restriction sites are near the dynamic mutation but whose action is methylation status-independent (BanI, EcoRI, PstI, BglIII, BclI and HindIII). Figure II.1 shows the restriction sites of a few enzymes near the site of the dynamic mutation and the hybridization sites of the most widely used probes.

Restriction fragment length depends on the number of nucleotide base pairs between the restriction sites of the enzymes used. It is estimated by the fragment's electrophoretic migration (the distance travelled being compared to that travelled by a fragment of known size) and is expressed in kilobases. Mutations are characterized by the difference between the length of the detected fragment and the size of the fragment normally isolated (which depends on the choice of enzymes). This difference, which is referred to as "delta", can be converted into a total number of trinucleotide repeats, taking into account the median of 29 trinucleotide repeats observed for normal alleles.

The combined use of methylation-sensitive and methylation-insensitive enzymes not only makes it possible to estimate the length of the alleles of the FMR1 gene, but also to determine the methylation status of the gene's promoter region. Additional cleavage of the fragments by methylation-sensitive enzymes does not occur in the presence of a methylated CpG island, i.e., for genes carried on the inactive X chromosome in females and in the case of full mutations. The images obtained after electrophoresis and hybridization (hybridization profiles) are therefore complicated and differ according to gender and the type of mutation.

Because X chromosome inactivation is random, females normally exhibit two electrophoretic bands, one corresponding to the inactive-chromosome fragment, the other to the shorter, active-chromosome fragment. In female premutation carriers, four bands may be visualized, since, in addition to the two normal fragments, the mutated allele is present both in the active and inactive form. However, in the case of the preferential inactivation of one of the alleles, only two fragments can be isolated, the active mutated allele and the inactive normal allele or vice versa (Rousseau et al., 1992).

cDNA probes are chosen on the basis of their position in relation to the restriction sites of the enzymes to be used so that the fragment containing the mutation can be revealed. Incorporating radioactive nucleotides into the probes permits visualization of the electrophoretic bands by autoradiography. More recently, the use of chemiluminescently labelled probes was proposed, but this approach would need to be validated more thoroughly (Gold et al., 2000).

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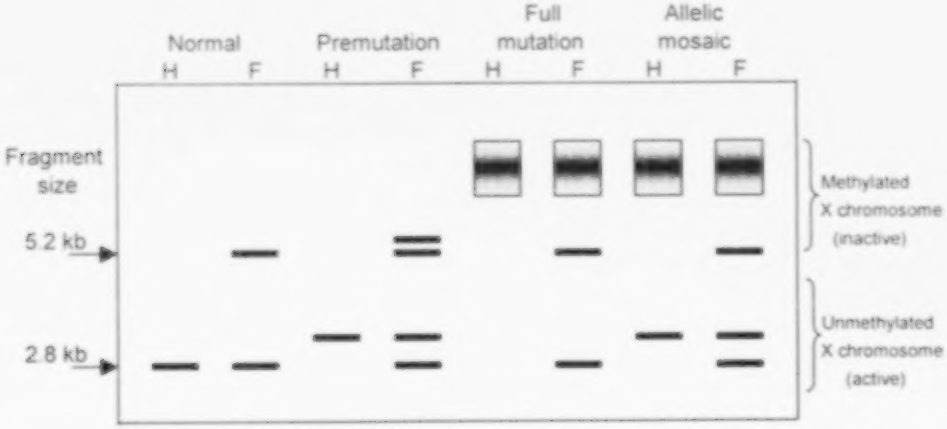
[185] Rousseau et al. (1994) summarize, in tabular form, all of the methods used by different authors up to 1994.

Figure II.1: Schematic representation of the fragment of the FMR1 gene surrounding the dynamic mutation site


Normal
Premutation
Full mutation
Allelic mosaic
H
F
H
F
H
F
H
F
Fragment size
5.2 kb
2.8 kb
Methylated X chromosome (inactive)
Unmethylated X chromosome (active)

Source: Inspired by Oostra et al. (1993) and Warren and Nelson (1994).

Legend: *5.2-kb fragment obtained by EcoRI enzyme digestion with 1) the main restriction sites, including the restriction sites of the enzymes sensitive to methylation of the gene's promoter, which is represented by the circle; 2) the first exon of the gene, which is represented by the rectangle and which includes the trinucleotide repeat; and 3) the main probes used in the Southern method (A = Pfxa3 = Ox0.55 = PX6; B = StB12.3 = pP2 = pfxa7; C = Ox1.9; D = pE5.1 = Pfxa1).*

Table II.1: Size of fragments (in kilobases) obtained by the Southern method for normal, premutated and mutated alleles according to the restriction enzyme(s) used

	EcoRI				**PstI**	**BglII**
		Males	**Females (active X)**	**Females (inactive X)**		
Normal allele	5.2	2.8	2.8	5.2	1.0	12
Premutation		2.9 – 3.3	2.9 – 3.3	5.3 – 5.7	1.1 – 1.6	
Full mutation	> 5.7	> 5.7	> 5.7	> 5.7		> 12.5

Source: Nussbaum and Ledbetter (1995).

The quality of the resolution (i.e., the separation of the bands corresponding to alleles of different sizes) depends on the length of the normal fragment and on the electrophoretic conditions. Thus, protocols yielding small fragments will permit better discrimination between normal alleles and small premutations, since delta is relatively large in relation to the fragment's normal size. However, such conditions will also promote considerable separation of heterogenous full mutations (somatic mosaics), which will result in a smear of weak intensity that is difficult to visualize. On the other hand, protocols based on a large, normal fragment and less resolution between alleles of different sizes make for better visualization of heterogeneous full mutations, which migrate in the form of more condensed bands.

Although no one protocol can be recommended for detecting all alleles, the protocol most often used as a first recourse seems to be that described by Rousseau et al. (1991a), namely, double digestion with the enzymes EcoRI and EagI and with the probe StB12.3 (Oostra et al., 1993; Rousseau et al., 1994; Nussbaum and Ledbetter, 1995). This protocol has the advantage of enabling one to determine the methylation status of the CpG island and of yielding intermediate-sized fragments suitable for identifying most alleles. However, it is preferable to perform an additional analysis for a differential diagnosis between normal and premutated alleles and to determine their size, either by an additional digestion with PstI (with the probe Pfxa3, as per the method of Yu et al., 1991) or with BclI, or by PCR, which is now the most commonly used approach (Rousseau et al., 1992; Snow et al., 1993; Nussbaum and Ledbetter, 1995). Similarly, heterogeneous full mutations can be condensed and thus better visualized by digestion with the enzyme BglII (Rousseau et al., 1992; Oostra et al., 1993; Murray et al. 1997).

Table II.1 (Nussbaum and Ledbetter, 1995) summarizes the size data on the fragments obtained with these different protocols according to gender and mutation type. Figure 3.1 shows the usual migration of the electrophoretic bands for double enzyme digestion with EcoRI + EagI.

II.3 PCR

With polymerase chain reaction (PCR), or *in vitro* selective DNA amplification, one can, starting with a trace amount of DNA, multiply the number of copies of a DNA segment contained between a pair of oligonucleotide primers. The primers anneal to specific DNA sites and, thanks to a DNA polymerase (Taq DNA polymerase or other enzymes with comparable activity), the multiplication of the DNA segment occurs logarithmically at the beginning, then peaks because of a lack of substrate. The exponential phase of the reaction can be explained by the fact that each DNA segment synthesized by the DNA polymerase serves, after its two strands are separated by heat denaturation, as a template for the synthesis of two more strands. A thermal cycler is used to program the denaturation, annealing and extension cycles at precise time intervals and at precise temperatures. The amplification products are then separated by gel (usually agarose or polyacrylamide) electrophoresis. Lastly, several techniques are used to visualize the amplification products.

PCR can be performed in 24 to 48 hours and requires only a trace amount of DNA, which permits a direct analysis (without prior cell culture) on an amniocentesis specimen, on a buccal smear or on a specimen from a Guthrie card. In addition, more specimens can be analyzed simultaneously than with the Southern method. Each electrophoretic gel should include at least one positive control (containing the DNA of an affected individual whose expansion size is known), two negative controls (one without DNA, the other containing the DNA of a subject who does not have the mutation of interest) and a molecular weight marker for estimating the size of the amplification fragments.

Since this is a quicker and less labour-intensive method than the Southern method and generally lends itself better to wide-scale use, a number of researchers have attempted to adapt PCR to diagnosing fragile X syndrome, by selecting primers flanking the trinucleotide repeat. Normal alleles and premutations[186] are amplified with this method, and their number of repeats can be accurately determined, while with the Southern method, which has a lower resolution capacity, one cannot clearly distinguish between these alleles. PCR was therefore adopted very quickly, as a complement to the Southern method, for the differential diagnosis between normal alleles and small premutations. However, during the first attempts, it was found that amplifying large expansions, which are especially rich in cytosine and guanine, was difficult (Fu et al., 1991). Two radically different options were then explored: devising variants of the basic technique for forcing the amplification of large expansions (Pergolizzi et al., 1992; Erster et al., 1992; Brown et al., 1993; Chong et al., 1994; Levinson et al., 1994; Abd El-Aleem et al., 1995; Nanba et al., 1995; Hilbert and Sabine, 1996; Hećimović et al., 1997; Houdayer et al., 1999), and possibility exploiting the nonamplification of an allele as a pointer for screening for mutations (Cao et al., 1994; Wang et al., 1995; Haddad et al., 1996; Larsen et al., 1997; Hamdan et al., 1997).

In the first option, the amplification problems can be partially overcome by replacing one of the nucleotides, dGTP, with 7-deaza2'-dGTP in the PCR reagent mixture (Pergolizzi et al., 1992; Erster et al., 1992). However, with this procedure, the ethidium bromide staining is not sufficient for visualizing the amplification products[187], which means that more complex and labour-intensive visualization methods must be used. Incorporating radioactive nucleotides (Fu et al., 1991; Levinson et al., 1994; Hilbert and Sabine, 1996) or chemiluminescently labelled primers (Abd El-Aleem et al., 1995; Nanba et al., 1995) is one of the proposed solutions, but many researchers prefer to use a combination of PCR and the Southern method (Pergolizzi et al., 1992; Erster et al., 1992; Brown et al., 1993; Abd El-Aleem et al., 1995; Nanba et al., 1995; Hećimović et al., 1997). *In vitro* selective DNA amplification, as described earlier (PCR), and electrophoretic separation are then followed by transfer and immobilization of the amplimers on a membrane (Southern blot) and by hybridization with a probe (a synthetic oligonucleotide) corresponding to 5-11 trinucleotide repeats, $(CGG)_{5\text{-}11}$. These probes can be radioactively (Pergolizzi et al., 1992; Erster et al., 1992) or chemiluminescently (Brown et al., 1993; Abd El-Aleem et al., 1995; Nanba et al., 1995; Hećimović et al., 1997) labelled in order to reveal the hybridization products. The other option consists in adjusting the experimental conditions to facilitate the exclusive amplification of normal alleles (Cao et al., 1994; Wang et al., 1995) or normal and premutated alleles (Haddad et al., 1996; Larsen et al., 1997; Melis et al., 1999) at the expense of that of longer alleles. Proceeding by exclusion, this analysis should, if no normal alleles are visualized, be followed by the Southern method.

Over the past few years, a number of researchers have relied on the use of a DNA polymerase that is more stable at high temperatures to improve denaturation and therefore amplification of the trinucleotide expansion (Chong et al., 1994; Larsen et al., 1997; Hećimović et al., 1997; Hamdan et al., 1997; Houdayer et al., 1999). Some of them have also made use of automated amplification fragment size reading, owing to the detection of fluorescent primers by a sequencer, in order to enhance gel resolution and improve reproducibility when estimating the size of amplified fragments (Larsen et al., 1997; Hamdan et al., 1997; Houdayer et al., 1999; Melis et al., 1999). These two options have also been used to develop a diagnostic kit[188], which is now commercially available for research purposes (Applied Biosystems, 2000).

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[186] The largest expansion that can be amplified varies according to the particular features of the technique used, but small premutations are always amplified, with the result that the distinction between a normal allele and a premutated allele can be made.

[187] Only a few teams have attempted to modify the experimental conditions in order to be able to visualize the amplification fragments with ethidium bromide after agarose gel electrophoresis (Cao et al., 1994; Chong et al., 1994).

Most authors agree that, regardless of the variant considered, there persists a major problem due to the fact that the preferential amplification of small alleles can compromise the identification of full mutations[189]. This results in false negatives in males with allelic mosaics (Erster et al., 1992; Snow et al., 1993; Brown et al., 1993; Haddad et al., 1996) and in females with full mutations, especially in prenatal diagnosis (Abd El-Aleem et al., 1995; Brown et al., 1996). In the presence of an allelic mosaic, the preferential amplification of shorter alleles can lead to an incorrect diagnosis of a premutation. Furthermore, false negatives can occur in cases of an allelic mosaic consisting of normal alleles in addition to a full mutation (Haddad et al., 1996). Such cases have been described but seem to be rare (Snow et al., 1993; van den Ouweland et al., 1994; Rousseau et al., 1994; Nolin et al., 1999; Mingroni-Netto et al., 1996; Haddad et al., 1996; Perroni et al., 1996; Milà et al., 1996; Orrico et al., 1998; Schmucker and Seidel, 1999; Gold et al., 2000). In females, the presence of a single, normal-sized allele can result from the nonamplification of a full mutation or from the presence of two identical alleles, since about 20% of the normal population is homozygous for one of the alleles of fewer than 54 CGG triplets (Brown et al., 1993). Consequently, PCR must be followed by the Southern method in several situations: when no alleles are amplified in a male; when a single allele is detected by PCR in a female; if the band intensity is too weak; and in cases of a large premutation or a small full mutation for determining the gene's methylation status. Some authors also recommend confirming the diagnosis with the Southern method when a premutation has been identified, in order to reduce the likelihood of making an incorrect diagnosis (false negative) in the presence of an allelic mosaic (Haddad et al., 1996; Houdayer et al., 1999), but this position is not taken by all authors (Abd-El-Aleem et al., 1995; Hećimović et al., 1997).

Also, none of the variants described above provide any information about the methylation status of the CpG island, which is essential for differentiating large premutations from full mutations. Several attempts have been made to determine the gene's methylation status by amplifying, by PCR, a sequence of the CpG island adjacent to the trinucleotide expansion (Wang et al., 1995; Das et al., 1997; Strelnikov et al., 1999; Weinhaeusel et al., 2000). The information obtained from such an approach, together with that obtained by amplifying the trinucleotide repeat, could meet the need for a screening tool. However, these approaches are still in the research stage, and their performance still needs to be documented.

Lastly, a few researchers have developed methods for extracting DNA from samples of dried blood and/or adapted their PCR technique to this type of specimen in order to make these techniques more suitable for wide-scale use (Holden et al., 1995a; Larsen et al., 1997; Hong et al., 1999; Strelnikov et al., 1999), for this type of sample can be collected by several different methods and is much easier to store, keep and transport.

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[188] Thus far, the results of only one study of the validity of this diagnostic kit have been published, as an abstract. This study involved a limited number of samples and did not cover the complete array of alleles (Brown, 2000).

[189] According to Brown et al. (1996), up to 10% (13% in prenatal diagnoses) of full mutations are refractory to amplification.

In short, from the original technique described by Fu et al. (1991), numerous variants have been developed, with each researcher attempting to improve the amplification of the trinucleotide expansion (especially of longer alleles), the fragment separation or visualization, or facilitate wide-scale use of the test. The resolution in PCR protocols depends on the electrophoretic gel used and on the amplimer visualization methods[190]. A high degree of accuracy is not essential for large expansions because of their mitotic instability, provided a distinction can be made between premutations and full mutations on the basis of the methylation status of the CpG island, but is extremely important for distinguishing between small premutations and normal alleles. The tests best suited for wide-scale use are those which are not based on a radioactive method, which do not require transfer onto a membrane or hybridization, and in which each sample requires a limited amount of handling.

The multitude of approaches that have been proposed are, in a way, indicative of the lack of consensus as to what the optimal approach is, which probably reflects the fact that most of these variants have not been adequately validated. Indeed, several techniques have been published, even though they still present unresolved technical problems (Chong et al., 1994; Nanba et al., 1995; Hećimović et al., 1997). Others have been used only in a limited number of individuals, or the results are not clearly presented for all the individuals tested (Pergolizzi et al., 1992; Erster et al., 1992; Chong et al., 1994; Levinson et al., 1994; Hilbert and Sabine, 1996; Hećimović et al., 1997; Holden et al. 1995a; Strelnikov et al., 1999; Brown, 2000). The complete array of alleles is not always covered in the tested individuals (Cao et al., 1994; Larsen et al., 1997; Hamdan et al., 1997; Strelnikov et al., 1999; Melis et al., 1999; Brown, 2000). Furthermore, some authors do not perform a systematic confirmation, given that the Southern method is applied straightaway on a selective basis according to the PCR results (Brown et al., 1993; Holden et al., 1995b), which can introduce bias into the test evaluation. Lastly, only three authors mention that the tests were interpreted in a blinded manner, i.e., without knowing the confirmation test results (Wang et al., 1995; Haddad et al., 1996; Larsen et al., 1997).

Several authors also report that this method is sensitive to the experimental conditions (Erster et al., 1992; Levinson et al., 1994; Larsen et al., 1997), but rare are those who provide data concerning the estimate of amplimer size from the standpoint of their accuracy and reproducibility (Larsen et al., 1997). Also, as in the Southern method, gel interpretation, which is based on a visual assessment of the bands revealed after electrophoresis, is subject to a certain amount of subjectivity and to a margin of error that could possibly affect discrimination between different types of alleles (Hamdan et al., 1997). The more recent methods with automated detection might improve this situation. However, they require more sophisticated equipment.

Consequently, apart from the test's validity, which would need to be documented by a rigorous, peer-reviewed, comparative study (Levinson et al., 1994), instituting such a test at a clinical laboratory requires both the necessary expertise for developing the technique and ensuring its quality at the local level (calibration, performance checks, reproducibility, etc.), the expertise for interpreting the results, and undergoing an outside inspection on a period basic.

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[190] The accuracy with which amplimer size can be estimated depends on the electrophoretic gel used and on the visualization method. Polyacrylamide gels permit better resolution than agarose gels. Thus, for example, some protocols based on agarose gel electrophoresis can only detect 30- to 50-bp differences between alleles (Cao et al., 1994; Namba et al., 1995), whereas polyacrylamide gel, in the best experimental conditions, permits separation between two alleles one triplet apart (Fu et al., 1991; Wang et al., 1995).

Remarkably, among the researchers who have attempted to improve the PCR method, there is no general agreement either as to the indications for using PCR. While the use of PCR as a complement to the Southern method is, for determining the size of normal alleles and small premutations, a well-established practice (see Section 3.4), the use of PCR as a first recourse is the subject of debate. Certain technical variants apply to the first scenario (Levinson et al., 1994; Hamdan et al., 1997; Houdayer et al., 1999), while others have been specifically developed for performing a large number of tests, most of which will prove negative. Some authors see the possibility of using these tests in wide-scale screening, whether the screening of pregnant women (Erster et al., 1992; Brown et al., 1993) or neonatal screening (Erster et al., 1992; Brown et al., 1993; Holden et al., 1995a; Strelnikov et al., 1999)[191]. For others, this approach may also be suitable for the screening of developmentally or mentally retarded individuals and sometimes even for doing a preliminary screen of samples sent from a clinic (Erster et al., 1992; Brown, et al. 1993; Cao et al., 1994; Chong et al., 1994; Abd-El-Aleem et al., 1995; Wang et al., 1995). However, the more recent literature is more hesitant, since it stresses the use of the Southern method as soon as the syndrome is suggested by the family history or the phenotype (Haddad et al., 1996; Houdayer et al., 1999).

II.4 IMMUNOCYTOCHEMICAL ANALYSES OF FMRP

A radically different approach is based on examining the gene's expression by means of immunocytochemical analyses of FMRP (fragile X mental retardation protein). In 1995, Willemsen et al. (1995) developed a method based on the use of anti-FMRP monoclonal antibodies, which are detected by an indirect alkaline phosphatase technique. The alkaline phosphatase is visualized under a microscope after staining. Since this technique detects the production of the protein by blood cells, it can be used to identify cells with a full mutation, as those with a premutation yield the same stain as normal cells.

This is a rapid approach that requires only a few drops of blood. However, it requires identifying lymphocytes, the result being expressed as a percentage of lymphocytes expressing FMRP. It has also been used in prenatal diagnosis by chorionic biopsy (Willemsen et al., 1996) and by amniocentesis (Willemsen et al., 1997a). Other authors are experimenting with variants of this technique (Jenkins et al., 1999). More recently, Willemsen et al. (1999) adapted their technique for use on hair root samples. Since hair is of ectodermal origin, FMRP expression might be more similar to that of the brain than that obtained with blood cells.

The blood smear method has been compared by a few researchers to DNA tests. Willemsen et al. (1997b) conclude that there is no overlapping between the normal values in males and the values observed in the presence of a full mutation. They propose a cutoff point of 42%, for which the sensitivity and specificity would be 100%. Since the test's discriminating power is not as good for females, they believe that it would be indicated only for screening for mental retardation or learning disabilities in boys and possibly for neonatal screening, if the ethical issues relating thereto are resolved. De Vries et al. (1998c) used this method to screen 412 mentally retarded boys and identified two new cases of the syndrome. They point out that there are still unresolved technical problems, since, during the first round of analyses, 7.8% of the samples revealed fewer than 60% lymphocytes expressing FMRP. Lastly, Tassone et al. (1999) examined the correlation between DNA analysis, FMRP expression and the cognitive, physical and behavioural assessment in 80 affected individuals, including several high-functioning males. In their estimation, the test's predictive power is not as good as originally reported by Willemsen et al. (1997b), since in affected individuals who are not mentally retarded, more than 50% of the lymphocytes can express the protein. They conclude that the diagnosis should always be confirmed by DNA analysis, but that this test could prove useful for predicting mental retardation in males only, provided other studies elucidate the correlation between phenotype and FMRP expression. Kaufmann and Reiss (1999) feel that this approach, which is also an excellent research tool, should complement DNA analysis, not replace it.

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[191] Presently, a few techniques can be used only in males (Cao et al., 1994; Chong et al., 1994; Wang et al., 1995; Holden et al., 1995a; Haddad et al., 1996).

As for the hair root technique, the authors of the original study report that there was no overlapping between the normal values and the proportion of hairs expressing FMRP in mentally retarded males and females with a full mutation (Willemsen et al., 1999). Since the technique is rapid and inexpensive, these authors see a potential application for the screening of boys and girls with mental retardation or learning disabilities or even for population screening. However, these preliminary results need to be confirmed, and this in a wider array of cases.

Although these techniques seem very promising, their validation is not yet completed. The exact indications for their use will have to be adjusted in light of these results, whether they are used as screening tests or a complement to DNA tests providing prognostic indications.

II.5 SUMMARY

Up until 1991, confirmation of fragile X syndrome was based on the detection of a fragile site at Xq27.3 after a cell culture in specific media. This method was labour-intensive, and, despite persistent efforts to standardize the technique, there were still problems with fragile site detection and the diagnostic criteria. The main limitation of the cytogenetic method is that it does not reveal most premutations and does not detect all full mutations. Consequently, screening, in affected families, individuals at risk for transmitting the syndrome cannot be done, which can considerably limit the possibility of genetic counselling. In 1993, with confirmation of the existence of false positives and false negatives, general agreement was quickly reached over discontinuing cytogenetic diagnosis, with molecular diagnosis supplanting cytogenetic diagnosis of fragile X syndrome both in prenatal diagnosis and for diagnostic confirmation in affected individuals. However, in cases of mental retardation or developmental delay and in the absence of a known family history of the syndrome, karyotyping is still indicated for ruling out other chromosomal abnormalities.

It is generally recognized that the Southern method is the reference diagnostic tool for fragile X syndrome and that PCR serves as a complement to this tool in order to distinguish small premutations from normal alleles. The most widely used Southern protocol is that based on double enzyme digestion with EcoRI and EagI and the use of the probe StB12.3. This protocol can be used to determine the gene's methylation status and to determine the size of the allele (Rousseau et al., 1991). Several PCR protocols are currently in use, most of which are inspired by Fu et al. (1991).

Many researchers have proposed variants of PCR that are to be used on a first-recourse basis for the purpose of identifying normal alleles and which are intended for wider-scale use of the test, Southern method being necessary only to confirm the diagnosis if no normal alleles are visualized. Despite these efforts, two problems persist: the lack of well-validated methods for determining the methylation status of the FMR1 gene by PCR and the preferential amplification of small alleles, which can lead to false negatives in females and in males with allelic mosaics. Furthermore, the validation of these methods is less than satisfactory. In particular, the error rate should be determined by rigorous comparative studies based on a population with an array of alleles as broad as that in the target population. It should be recognized that, despite these weaknesses, PCR as a first recourse is an increasingly utilized approach (Holden et al., 1995a; 1995b; Brown et al., 1996; Meadows et al., 1996; Murray et al., 1996; de Vries et al., 1997; Gérard et al., 1997; Hećimović et al., 1998; Crawford et al., 1999; Ryynänen et al., 1999; Millan et al., 1999; Youings et al., 2000).

The approach based on examining the gene's expression by means of immunocytochemical analyses of FMRP using blood smears or hair roots seems promising, but its validation is not yet completed. A few technical problems still need to be resolved, and, depending on the indications chosen, this method could be used as a screening test or a prognostic tool.

APPENDIX III:

ACMG AND ACOG GUIDELINES

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Guidelines of the American College of Medical Genetics (ACMG, 1994)

Individuals for whom genetic diagnosis should be considered are as follows:

- Those of either sex with mental retardation, developmental delay or autism, especially if they also have:
  - characteristics of fragile X syndrome
  - a family history of the syndrome
  - relatives with mental retardation of unknown etiology
- Those seeking genetic counselling for reproductive purposes if they have:
  - a family history of fragile X syndrome
  - a family history of mental retardation of unknown etiology
- Fetuses of mothers known to carry a premutation or a full mutation
- Individuals who have a cytogenetic test result that is discordant with their phenotype.

The method of choice for testing is molecular analysis, which is sufficient in cases of a positive family history. However, if the etiology of the mental retardation is unknown, DNA analysis for fragile X syndrome should be accompanied by karyotyping. This approach is justified by the fact that constitutional chromosomal abnormalities are frequently identified in mentally retarded individuals referred for fragile X testing.

Guidelines of the American College of Obstetricians and Gynecologists (ACOG, 1996)

Individuals for whom genetic diagnosis should be considered are as follows:

- Developmentally delayed children and individuals with mental retardation of unknown etiology.
- Individuals seeking genetic counselling for reproductive purposes if they have:
  - a family history of fragile X syndrome
  - a family history of mental retardation of unknown etiology
- Fetuses of mothers known to carry a premutation or a full mutation.

The method of choice for testing is molecular analysis. However, when the fragile X test is negative in cases of mental retardation of unknown etiology, karyotyping should be performed to rule out a chromosomal abnormality.

APPENDIX IV:

ORGANIZATION OF REEDUCATION AND SOCIAL INTEGRATION SERVICES

APPENDIX IV: ORGANIZATION OF REEDUCATION AND SOCIAL INTEGRATION SERVICES

In this appendix, we briefly discuss the goals and policies underlying the changes that have occurred over the past few decades in services for the mentally impaired in the health and social services system and review the role of rehabilitation centres and the involvement of the other sectors of the system in this regard.

IV.1 GOALS AND POLICIES IN THE AREA OF SOCIAL INTEGRATION

In Québec, as in many Western countries, the 1970s were marked by an affirmation of human rights, including those of the handicapped, and by a willingness to deinstitutionalize the mentally impaired. The *Charter of Human Rights and Freedom*[192], which was adopted in 1975, gave rise to the Commission des droits de la personne, and *An Act to secure the handicapped in the exercise of their rights*[193] gave rise to the Office des personnes handicapées du Québec (OPHQ) in 1978. The OPHQ's mandate was to see to the coordination of the services dispensed to the handicapped, to provide information to them and to promote their interests. The objective was to foster their educational, occupational and social integration. To this end, the main intervention tool was to be the development of an individualized service plan.

In 1984, the OPHQ tabled a policy proposal (OPHQ, 1984), whose goals were subsequently adopted by the government. These goals stressed the importance of promoting the development of the abilities of the handicapped and of relying, for the purposes of preparing an individualized intervention plan, on an overall assessment of their abilities and potential. In 1988, the Ministère de la Santé et des Services sociaux incorporated these goals in its policy statement on integrating the mentally impaired (MSSS, 1988). In the area of deinstitutionalization, the objective is to gradually discontinue institutionalization for reasons of mental impairment only. The advocated goals place emphasis on promoting social roles, which requires developing individuals' abilities and skills, and on social integration. To achieve social integration, a range of services is made available, as well as mechanisms designed to ensure the consistency of service organization at the regional level.

While the objectives, in terms of deinstitutionalization, seem, in large part, to have been achieved for children[194], it is less obvious, as we shall see, that the network of available services completely meets the needs of the people concerned. The MSSS is in the process of reviewing its policy, and, to assist it, two studies have been carried out, a survey among the main players in the health and social services system (Larmarre, 1998) and a survey among the mentally impaired, their families and professionals in the field (Perreault, 1997)[195].

IV.2 REHABILITATION CENTRES

Rehabilitation centres have been in existence since 1971, but their mandate was revised in 1991 under *An Act respecting health services and social services*[196]. Rehabilitation centres are now distinguished according to their clientele.

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[192] R.S.Q., c. C-12 (Québec).

[193] R.S.Q., c. E-20.1 (Québec).

[194] As regards mental impairment (not mental or physical health), only 65 young people under the age of 18 managed by rehabilitation centres for the mentally impaired were institutionalized in Québec in 1997-1998.

[195] The findings of these two surveys are discussed in Section 5.4.

[196] R.S.Q., c. S-4.2 (Québec).

Since fragile X syndrome can first manifest as speech delay and developmental delay, and since mental retardation is generally documented only once the child has reached school age, two types of rehabilitation centres may be involved with these patients: rehabilitation centres for the mentally impaired and rehabilitation centres for the physically impaired.

The mission of rehabilitation centres for the mentally impaired (RCMIs) is to provide adaptation, rehabilitation and social integration services to the mentally impaired and to provide persons to accompany them and support services to their families and friends[197]. The objective of these interventions is to develop the abilities of the mentally impaired, to increase their autonomy, to improve their quality of life and promote their social integration and participation. RCMIs assess the individuals' needs and see that the services are provided, either at the rehabilitation centre, in the individuals' home or by other people or organizations capable of helping them. They therefore intervene in a complementary fashion with the other organizations[198] that can provide services, and often coordinate these services. To this end, they develop or participate in the development[199] of an individualized service plan, which should be reviewed on a regular basis to check that the services meet the individual's needs. While several organizations are involved in providing services, each develops an intervention plan and provides the services accordingly. In practice, the services dispensed directly by RCMIs consist mainly of family support and early stimulation for younger individuals, and, starting in adolescence, vocational counselling and residential living support (FQCRPDI, 1994).

Rehabilitation centres for the physically impaired (RCPIs) provide services to individuals who have a significant and persistent physical impairment that can cause functional limitations or disabilities and cause them to experience situations of handicap. Most of these individuals have a motor, visual, auditory, language or speech impairment or multiple impairments. This definition does not include people with a language or speech impairment resulting from mental retardation or psychopathological disorders (e.g., autism). Intended to serve a clientele with an array of impairments, RCPIs generally provide a vast range of services, including both functional reeducation (speech therapy, occupational therapy, etc.) and social integration/rehabilitation. The individualized service plan is developed by a multidisciplinary team, which may include a special education teacher, a social worker, a speech therapist, an occupational therapist and a psychologist.

This distinction based on the nature of the impairment can, in some cases, pose problems in terms of referrals. For example, the respective roles of rehabilitation centres with regard to young children with psychomotor developmental delay is unclear in some regions. The Fédération Québécoise des Centres de réadaptation pour les personnes présentant un déficience intellectuelle (FQCRPDI) and the Fédération de la réadaptation en déficience physique du Québec (FRDPQ) published, in 1996, joint goals concerning the response to needs of people who are both physically and mentally impaired (FRDPQ and FQCRPDI, 1996). Accordingly, it would be up to rehabilitation centres for the physically impaired to follow these children as long as severe signs of mental retardation have not been detected, in order not to label these children too hastily. Once signs of mental retardation become more obvious (generally after the age of 4 years), the children would be referred to an RCMI for evaluation, with, if necessary, the RCMI taking over from the RCPI. This agreement has been put into practice in only a few regions.

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[197] R.S.Q., c.S-4.2, s. 84 (Québec).

[198] These may be other organizations in the health and social services system, partners in other systems, community organizations or natural helpers in the family.

[199] Under Section 103 of *An Act respecting health services and social services* [R.S.Q., c.S-4.2, s. 103 (Québec)], the institution that dispenses most of services or a professional designated jointly by the resources concerned, must develop an individualized service plan (ISP).

Under the agreement concluded between the Ministère de la Santé et des Services sociaux (MSSS) and the Ministère de l'Éducation du Québec (MEQ) in 1990, a rehabilitation centre may be called upon to provide speech therapy, psychology and psychoeducation services in schools for children who are already enrolled at its institution (MSSS-MEQ, 1990). These measures were taken to prevent duplication and ensure continuity of the services for these children, but the application of this agreement has been found to vary considerably from one region to another. Also, it seems that RCPIs are most often the ones involved in providing RCMIs these services in schools, while RCMIs have a tendency to leave the responsibility for children who have reached school age[200] to the schools.

IV.3 THE OTHER SECTORS INVOLVED

Of the other sectors of the health and social services system involved in providing or coordinating reeducation or social integration services, mention should be made of hospitals and local community service centres (CLSCs). In hospitals, certain specialized clinics (development, pediatric neurology, child psychiatry or other clinic) refer patients for speech or occupational therapy in their institutions. Psychological services are usually used to complete the cognitive and functional assessment.

As part of their general mandate, CLSCs have a responsibility with regard to home care and family support, but the manner in which the services are organized differs considerably from one CLSC to another. For special patient populations, some CLSCs operate in a more or less centralized manner. The personnel specifically devoted to these populations—the nurses and social workers—is then well identified. This is sometimes the case for the mentally impaired clientele. Elsewhere, however, the mentally impaired may not be as privileged, since they may be lumped together with everyone else requiring home care and family support.

Two services that are especially important for the mentally impaired and their families are respite care and psychosocial support. Social workers also have an important role to play in referring users to other services, which includes the providing of complete information, a needs assessment and assistance in obtaining financial services and support. For families with a physically handicapped and/or mentally impaired child, the administrative procedures can be rather demanding, given that, for each request, the family must demonstrate its needs again, whether it is for obtaining home services, adapted transport, emergency respite, special lodging or OPHQ or RCMI services. The available financial support includes the tax credit, the special family allowance and various other allowances, including respite care and transportation and accommodation expenses during consultations with specialists.

For the mentally impaired, CLSCs are the point of entry of choice toward RCMIs, since their role has been defined in the mechanisms of access to this service. Referral to an RCMI requires a prior assessment of the individual by a CLSC and approval of the case by the regional authority in charge. These access mechanisms have been in place since the early 1990s, and although the administrative red tape involved is deplored by many professionals, collaboration between the CLSCs and RCMIs seems to have been enhanced as a result of instituting these mechanisms (Lamarre, 1998). Lastly, with the restructuring of RCMIs, professionals were transferred, in some regions, to CLSCs, a certain number of which can therefore also provide specialized services directly.

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[200] Throughout the school years, they have little contact with these families, except, sometimes, to provide educational assistance.

There are considerable differences in the services CLSCs provide to the mentally impaired. In part, these differences are due to the resources allocated to this clientele, to the organization of services, to the number of professionals involved, to their specific interests and to their more or less sustained involvement with this clientele. The nature of the collaborative efforts developed between the different partners[201] basically depends on the people in place.

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[201] In some cases, a CLSC can also serve as a liaison between the family and the school.

APPENDIX V:

ORGANIZATION OF EDUCATIONAL SERVICES

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In this appendix, we briefly review the legislation governing the organization of educational services, and the ideas and policies which have guided the changes that have occurred over the past few decades. We then take an overall look at the places where handicapped students and students with social maladjustments or learning disabilities (HSSSMLD) are educated.

V.1 THE LEGISLATION

Under the *Education Act*[202], every person is entitled to educational services up to the age of 18 years, or 21 years in the case of a handicapped person within the meaning of *An Act to secure the handicapped in the exercise of their rights*[203]. These educational services, which are described in the *Basic school regulation for preschool, elementary and secondary education*[204], include preschool education services, elementary and secondary instructional services, student services and special services. Student services include, among others, academic and vocational counselling, and psychological, speech therapy, remedial education, psychoeducational and special education services. Special services include home or in-hospital instruction for children who are unable to attend school because they require specialized health care or social services.

Under Section 234 of the *Education Act*, the school boards are required to adjust the educational services provided to handicapped students and students with learning disabilities according to their needs and in keeping with the requirements set out in the basic school regulation. After consulting the advisory committee on services for handicapped students and students with social maladjustments or learning disabilities[205] (HSSSMLD), the school board must develop and adopt a policy concerning the organization of educational services for such students. This policy must spell out both methods for integrating students into regular classes and support services for this integration and methods for grouping students together in special classes or schools. Methods for evaluating HSSSMLD and how intervention plans will be developed must be detailed in the policy as well.

It is the school principal's responsibility to establish an intervention plan adapted to the student's needs, but it must be prepared with the input of the student, his or her parents and the school personnel who will be providing services to the child[206]. The principal must also see that this plan is evaluated periodically. For handicapped children, some exemptions from the basic school regulation are allowed. Exemptions concerning compulsory subjects and the number of hours devoted to educational services are mainly granted to elementary and high school students with profound mental retardation and to high school students with moderate or severe mental retardation. For these students, an individualized program makes it possible to focus on the development of their skills in areas such as communication, motricity, socialization and functional autonomy. The MEQ has developed special educational programs for these students at the elementary level and, more recently, at the high school level. The programs for high school students have been in place on a trial basis since 1996, but they have not yet been put into general use.

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[202] R.S.Q., c. I-13.3, s. 1 (Québec), adopted in July 1989 and updated in 1999.

[203] R.S.Q., c. E-20.1 (Québec).

[204] R.S.Q., c. I-13.3, r. 3.1 (Québec).

[205] Under Sections 185 and 186 of the *Education Act*, each school board must form an advisory committee on services for HSSSMLD, which consists mostly of representatives of the parents of the students concerned. Its function is to give its opinion on the policy concerning the organization of educational services for HSSSMLD and on the allocation of financial resources earmarked for them.

[206] Under the new Section 96.14 of the version of the *Education Act* that has been in effect since October 1999 (previously Section 47).

In short, the school boards are required to provide services adapted to the needs of HSSSMLD, but they do have a certain amount of leeway with regard to the choice of services they offer, for two different modalities are provided by law: integrated services and segregated services. The boards determine which educational services are to be offered at which schools[207], and they can conclude agreements with other school boards or with private institutions for students with special needs that they are unable to meet[208].

In practice, until quite recently, the organization of services differed considerably from one school board to another, from the offer of a range of services to the exclusion of one of the two modalities. The revised version of the *Education Act*, which has been in effect since 1999, places greater emphasis on integrating these children into regular classes, since the school boards are required to organize the educational services for the persons under its authority and since they may not conclude service agreements unless they can demonstrate that they do not have the necessary resources or if they agree to grant the parents' request[209]. Furthermore, they must consult the students concerned and/or their parents, and the advisory committee on services for HSSSMLD[210].

V.2 SCHOOL INTEGRATION: GOALS AND POLICIES

The current situation is the result of considerable changes that have taken place since the early 1960s[211]. Previously, very few schools provided educational services for handicapped children. With the Parent Report (Royal Commission of Inquiry on Education in the Province of Québec, 1963-1966), emphasis was placed on the educational system's responsibility toward all children, including those with special educational needs, and although the report spurred the development of special services, the idea of sticking to regular education as much as possible whenever the child's condition so permitted had already been put forth. The COPEX Report (Ministère de l'Éducation du Québec, 1976) recommended instituting a range of services, from regular classes to special classes, with a series of intermediate support measures. This step-type system was to be flexible enough to allow students to go from one modality to another, with the most appropriate services to be determined on the basis of functional assessments. The ministerial policy of 1978 (Ministère de l'Éducation du Québec, 1978) supported school integration based on as normal an educational path as possible and stressed the double objective of education, which was to promote both learning and social integration. With the adoption of the *Education Act* in July 1989 and the updating of the Ministry's 1992 policy on special education (Ministère de l'Éducation du Québec, 1992), another trend started, since preference was now being given to adapting services to the children's needs rather than to adapting children to the different types of services available.

These changes were, of course, part of a broader movement in which knowledge[212], concepts and rights were evolving. With respect to rights, we previously mentioned that the Québec government adopted the *Charter of Human Rights and Freedoms* in 1975 and *An Act to secure the handicapped in the exercise of their rights* in 1978. As for concepts, those of handicap[213], and mental impairment[214] and the very concept of school integration have changed significantly. The concept of normalization[215], which came into being in Scandinavia, gave rise, in North America, to two different interpretations: mainstreaming and inclusion[216]. The main trend, mainstreaming, is the offer of a range of integrated and segregated services, although integration is encouraged as much as possible, while inclusion implies more-systematic integration, with resources transferred from special services to regular teaching. These differences still translate into the position adopted by a number of lobby groups[217], including certain parents' associations[218] (e.g., Association québécoise pour l'insertion sociale), which demand the systematic integration of handicapped children into regular educational settings. Many complaints have been filed with the Commission des droits de la personne et de la jeunesse, and several cases have been filed with and/or heard before the courts, most of which concern mentally retarded children. The plaintiffs' arguments are based on the infringement of the right to equality, specifically, on discrimination based on handicap, which is in violation of Québec's *Charter of Human Rights and Freedoms* and the *Canadian Charter of Rights and Freedoms*[219].

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[207] Section 236 of the *Education Act*.

[208] Section 213 of the *Education Act*.

[209] Section 209 of the *Education Act*.

[210] Section 213 of the *Education Act*.

[211] For a more detailed discussion of the background to the educational policies, see, for example, Garon (1992), OPHQ (1995) or Conseil Supérieur de l'Éducation (1996).

[212] As regards knowledge, consider, for example, the evolution of diagnostic and management modalities, the development of special reeducation services, the experiences with early stimulation, and the innovations in individualized and personalized education.

The debate over educating handicapped children is therefore not over. After the initial enthusiasm over the integration project, there were difficulties carrying it out, and reservations were expressed. In 1996, the Conseil supérieur de l'Éducation (CSE) summarized the obstacles to success, calling attention to the following: 1) the inadequate preparation of school personnel; 2) the inadequate support given to teachers in regular classes; 3) the method of funding for HSSSMLD; and 4) the inadequate planning and especially the inadequate evaluation of the interventions in these children. As for the reservations, mention should be made of the following arguments: 1) the incompatibility between the quality of education for all and equal opportunity; 2) no rigorous demonstration that the integrated model is superior to the segregated model or vice versa; and 3) the fact that the quality of integration depends on the resources devoted to it (Conseil supérieur de l'éducation, 1996). A number of authors agree with this latter observation, and the CSE goes as far as saying that "actually, the conditions for success are met to such a small degree that they jeopardize the very principle of integration".

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[213] With the classification developed by Wood for the WHO in 1980, the consequences of diseases are considered from several vantage points: impairments, disabilities and handicaps. This distinction introduces the role of the environment into the development of a handicap and leads to the identification of the individuals' needs from a social integration perspective (INSERM, 1990).

[214] The evolution of the concept of mental retardation, or mental impairment, is summarized in the American Association on Mental Retardation manual (1992). Originally, the definition was based exclusively on IQ, but the notion of impairment in adaptive behaviour was incorporated in 1959. The latest definition specifies ten adaptive skill areas that can be affected by a limitation in intellectual abilities. It is, therefore, a functional definition of mental impairment that attempts to identify the individuals' needs and the support they require for performing their daily tasks in their personal and social environment.

[215] The aim of normalization is to provide services within as normal a possible social framework.

[216] For a more detailed discussion, see Doré et al. (1996).

[217] For a summary of the positions of the various stakeholders in the school sector and of the lobby groups, see Tables 2 (pp. 114-115) and 3 (pp. 115-116), respectively, in Doré et al. (1996).

[218] This position does not reflect the opinion of all parents. According to Bouchard et al. (1996), the parents most in favour of integration into regular classes are, at the elementary level, those whose children have good socialization behaviour skills, and, at the high school level, those whose adolescents are more autonomous.

[219] Schedule B of the *Canada Act, 1982* (UK), 1982, c. 11.

Although the type of integration currently preferred is an environmental model[220], which is based essentially on adapting the handicapped students' environment, the CSE's conclusions are in favour of what is referred to as an "anthropologic" model, which is based on interaction between the HSSSMLD and their environment and which requires reciprocal adaptation. According to the CSE, what is essential is therefore "to strike a balance between responding to the needs of certain students and taking the school system's limits into account", since integration at any cost is not indicated for all students or in all circumstances. The ministerial policy on special education was therefore updated in 1999 (Ministère de l'Éducation du Québec, 1999b) in order to reflect educational reform and the amendments made to the *Education Act*. Although school integration is preferred, it is clearly conditional on the existence of benefits for the student and/or the absence of major constraints on his or her environment[221].

V.3 THE PLACES OF EDUCATION OF HSSSMLD

Although the policies and laws of the past 20 years have promoted the integration of HSSSMLD into regular schools, there is an entire range of educational services, from regular classes to special schools. For students spending more than 50% of their time in regular classes, support is provided, either to the student and to the teacher in the classroom, or by students spending several hours a week in a resource class. Placing students with disabilities in special classes in regular schools is an intermediate solution. Such classes are either heterogeneous or homogeneous in terms of the type of handicapped children or children with disabilities in them. Lastly, school boards may call upon a certain number of special schools, often private but recognized by the public system. These schools, which are generally intended for specific populations, accept children with autism, learning disabilities and mental retardation with or without behavioural problems.

For a general idea of the places of education of fragile X children, we had to examine MEQ's data concerning the education of HSSSMLD and see which categories of HSSSMLD fragile X children can be identified with. The classification of HSSSMLD used by the educational system makes a first distinction between students with disabilities and handicapped students (Table V.1). Students with mild mental impairment are counted as part of students with social maladjustments or learning disabilities, while those with moderate to severe or profound mental impairment are considered to be handicapped students. Under the heading of multiple impairements there are several categories involving a combination of mental impairment and other impairments or behavioural problems.

Fragile X students can, in fact, be found in several of the above-mentioned categories. Depending on their age and dominant symptoms, they can be classified with students with isolated mental impairment (mild, moderate to severe, and profound), but the less symptomatic can also be found with students with social maladjustments or learning disabilities (mild or severe). If behavioural problems dominate the clinical picture, these children are no doubt placed in the behavioural problems or multiple impairments category. Lastly, it is not impossible that a few children are labeled autistic (classified among those with severe developmental problems), although usually, all the diagnostic criteria for autism are not met.

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[220] See Conseil supérieur de l'éducation (1996) for a discussion of the existing conceptual models and their influence on educational pratices in different countries.

[221] Under Section 235 of the *Education Act* (Québec, 1999c), a student must be integrated into a regular class if it has been established, on the basis of an evaluation of his or her abilities and needs, that such integration would facilitate his or her learning and social integration and would not impose an excessive constraint or significantly undermine the rights of the other students. In addition, under Section 96.14, the school boards are now required to evaluate the students' abilities and needs prior to placement.

Table V.1: Classification of HSSSMLD used by the MEQ

Handicapped children	**Children with social maladjustments or learning disabilities**
Moderate to severe mental impairment	Mild learning disabilities
Profound mental impairment	Severe learning disabilities
Motor impairment (mild/severe)	Behavioural problems
Organic impairment	Mild mental impairment
Visual impairment	
Auditory impairment	
Multiple impairments	
Severe developmental problems	

The MEQ's classification differs from that advocated by the Office des personnes handicapées du Québec (OPHQ), according to which mildly mentally impaired children are considered handicapped. This difference can, according to the OPHQ (1995) and the Conseil supérieur de l'éducation (1996), deprive a certain number of these students of free educational services between the ages of 18 and 21 years, since only students recognized as being handicapped within the meaning of *An Act to secure the handicapped in the exercise of their rights* can receive these services up to the age of 21. The MEQ would act in such a way so as not to put these students at a disadvantage as result of hasty labelling, given the relative inaccuracy of the measurement instruments. It should be noted that the MEQ's definitions are based on the development quotient[222] as opposed to the IQ (in addition to limitations in adaptive functioning), which may make it more difficult to identify mild retardation during the early years.

According to MEQ statistics for the years 1996-1997 and 1997-1998, about 1.30% of the public school population at the preschool, elementary and secondary school levels would present a handicap and about 11%[223] of the students would present social maladjustments or learning disabilities (Ouellet, 1997; MEQ, 1999b). According to 1996-1997 data, about 0.6% of students have isolated mental impairment, 58% with mild retardation, 30% with moderate to severe retardation and 12% with profound retardation (Ouellet, 1997). However, approximately 80% of the students for which the statistics indicate multiple impairments are mentally impaired as well (CSE, 1996), which brings the percentage of mentally impaired students to 1.13% of the entire school population[224] and to 9.25% of HSSSMLD (Ouellet, 1997).

The Conseil supérieur de l'éducation (1996) points out that these statistics, which are derived from the school population statement, should be viewed with caution, given that it is submitted mainly for funding purposes and that beyond a certain ceiling, the financial benefits of declaring more HSSSMLD is negligible for the school boards. Therefore special-needs students in the public sector are probably undercounted, and this more so for mild mental impairment than more severe and more pronounced forms in younger students, the proportion of HSSSMLD identified being higher at the high school level than at the elementary and preschool levels[225].

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[222] The development quotient is the ratio of the developmental age to the chronological age.

[223] Statistics on the proportion of students with special educational needs differ substantially (1 to 17% in 1990) from one country to another because of different classifications and counting methods (CSE, 1996).

[224] This estimate is very close to the American data for 1993. According to U.S. Department of Education statistics, 1.14% of children aged 6 to 17 years would present mental impairment (cited by Battaglia et al., 1999 and King et al., 1997).

As for the places of education, public sector statistics for 1996-1997 for children with isolated mental impairment provide the following indications (Ouellet, 1997). At the preschool level, most children with mild or moderate to severe mental impairment are integrated into regular classes (78% and 60%, respectively[226]), whereas, nearly 50% of children with profound mental impairment attend a special school, with more than 20% receiving their education in reception centres, hospitals or at home. Few places are available in special schools, which report that there are waiting lists at the preschool level. At the elementary level, there are several educational modalities. About a third of children with mild (37%) or moderate to severe (26%) mental impairment are placed in regular classes. However, most such children are in special classes, which are usually heterogenous, less often homogeneous. The proportion of children in a special school increases with the severity of the mental impairment, it being more than 50% in the case of children with profound mental impairment. Lastly, about 5% of children with profound mental retardation receive their education in reception centres, hospitals or at home. At the high school level, the proportion of students with mental impairment integrated in regular classes barely exceeds 5% (7.8%, 3.9% and 0.9% for mildly, moderately and profoundly mentally impaired students, respectively), and most of them are in special classes, except those with profound mental impairment, who mainly attend special schools. Very few handicapped students are in vocational training, and the proportion of those who graduate is low[227].

The statistics given in the last paragraph only concern students in the public school sector with isolated mental impairment and probably overestimate, to a certain degree, the actual integration rate, since the coexistence of other impairments changes the educational profile. Thus, for example, at the high school level, most students with moderate to severe mental impairment and another impairment are educated in special schools (Ouellet, 1997). This trend is also documented in children with both behavioural problems and mental impairment (Conseil supérieur de l'éducation, 1996). As for the private sector, there are institutions that do not accept handicapped children and others specifically devoted to such children. If this sector were taken into account, the proportion of children attending special schools would probably be higher (especially among students with more severe impairments), but students with mental impairment attending schools in the private sector would account for only about 2% of all mentally impaired students (Ministère de l'Éducation du Québec, 1999b).

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[225] For example, in 1996-1997, the proportion of students with isolated mental impairment was 2.6‰, 5.6‰ and 8‰ at the preschool, elementary and secondary levels, respectively (Ouellet, 1997).

[226] The partial data that we have for 1997-1998 indicate progress in regular-class integration at the preschool level, with rates of 83% and 71% for students with mild or moderate to severe mental retardation, but relatively stable rates for the other levels of education (Ministère de l'Éducation du Québec, 1999b).

[227] Yet, integration into the job market, which is especially problematic in the presence of associated behavioural problems, seems to be facilitated by vocational training and participation in workplace apprenticeships (Ministère de l'Éducation du Québec, 1999b).

Apart from the type of school, accessibility to student services has been examined from the standpoint of the human resources working with HSSSMLD. They include special education teachers (remedial teachers), reeducation and psychosocial intervention professionals, and support staff (special education technicians, handicapped student attendants, etc.). Among these personnel at the preschool and elementary levels, there has been, since the early 1990s, a decrease in the number of special education teachers, who tend to be replaced by support staff. Also, professional personnel are being assigned more and more to an increasing number of schools (Conseil supérieur de l'éducation, 1996). At the high school level, a survey comparing the personnel in school boards that integrate HSSSMLD and those in all the school boards also showed a different resource distribution, with fewer special education teachers when there is more integration and a few more professionals serving as support staff for regular teachers (Boucher and Poulin, 1995). It was also found that the professionals' tasks were fragmented.

According to data provided by the Ministère de l'Éducation du Québec (1999b), the number of student services personnel increased in terms of full-time equivalent positions between 1990-1991 and 1997-1998 for each job category directly involved in services for HSSSMLD. Although this increase is proportionately greater for special education technicians and remedial teachers, the absolute number of technicians largely exceeds the number of other professionals[228]. Furthermore, the number of psychologists, psychoeducators and social workers decreased during the second half of the 1990s but did not return to the 1990-1991 level. In its 1999 policy on special education, the Ministère de l'Éducation du Québec (1999b) acknowledges that "the budget cuts in the field of education over the past few years may have had an impact on services for HSSSMLD, even if the funds allocated to these students were not directly affected by these cuts". In its action plan on special education, the Ministère de l'Éducation du Québec (1999a) plans to increase the number of professionals and teaching support staff for students and teachers, especially in the areas of special education, psychology, psychoeducation and speech therapy. The Ministry also promises to reduce the administrative red tape for enrolling HSSSMLD and to review the funding methods.

Overall, therefore, more handicapped students are being integrated into regular classes than in the past, although the integration of mentally impaired and multiply impaired students is proceeding more slowly than for other types of impairments. The type of education chosen depends a great deal on the educational level, the severity of the impairment and the choices made by the school boards. There are, in fact, very significant regional differences (Garon, 1997). Lastly, whatever the places of education, accessibility to student services provided by qualified personnel is necessary for meeting the special needs of HSSSMLD (Conseil supérieur de l'éducation, 1996).

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[228] For example, the tentative number of full-time-equivalent positions for 1997-98 was about 1,822 for special education technicians, as opposed to 102, 210, 308, 608, 21 and 10 for remedial teachers, speech therapists, psychoeducators, psychologists, social workers and occupational therapists, respectively (Ministère de l'Éducation du Québec, 1999b).

APPENDIX VI:

PROACTIVE HIGH-RISK-POPULATION DIAGNOSTIC AND SCREENING STRATEGIES

APPENDIX VI: PROACTIVE HIGH-RISK-POPULATION DIAGNOSTIC AND SCREENING STRATEGIES

In this appendix, which is provided as a supplement to Section 6.1.3, we briefly review the experience accumulated in various parts of the world in terms of proactive fragile X diagnosis and screening. We shall not look at all of the older studies that exclusively concern the diagnosis of the syndrome in individuals institutionalized for mental retardation. However, we will examine the main studies, generally more recent, which involve a broadening of the indications, more-diversified recruitment of the at-risk population, clinical objectives (clinical follow-up, genetic counselling) and, in some cases, the discontinuation of cytogenetic tests in favour of molecular tests. The purpose of this appendix is not to provide an exhaustive account of these studies or a detailed discussion of their results, but rather to show the extent of this experience and the diversity of the approaches used.

Table VI.1 provides a synopsis of four studies carried out in different European countries involving mentally retarded individuals (Gustafson et al., 1986; Kähkönen et al., 1987; Gabarron et al., 1992) or individuals with learning disabilities (Tranebjaerg et al., 1994). The objective of these studies, all of which were based on cytogenetic analyses, was to determine the prevalence on a regional basis. Only the Spanish study (Gabarron et al., 1992) had service-related objectives as well, providing a clinical follow-up of the diagnosed cases and genetic counselling for the families. The method for recruiting the at-risk population varied considerably from region to region, depending on the availability of registers (registers of mentally retarded individuals, registers of students with learning disabilities) or survey data (mental retardation screening in schools). In Denmark, the term *mental retardation* is avoided, with the result that all children with special educational needs are considered as having "learning disabilities", whether they are mentally impaired or not (Tranebjaerg et al., 1994).

Table VI.2 summarizes the experience acquired in three regions of Great Britain. All of these studies involved young people with learning disabilities recruited in various institutions: special schools (Webb et al., 1986; Slaney et al., 1995; Youings et al., 2000), regular schools (Murray, 1996; Youings et al., 2000) and/or hospitals or residential facilities for the mentally retarded (Webb et al., 1986). In Great Britain, children with special education needs are systematically screened for by means of academic performance tests administered at the age of 7 years. Thus, as in Denmark, it is a heterogeneous population, as it includes children who are and who are not mentally retarded. The identification of special educational needs seems to facilitate this population's access to services and lends itself to the study of the prevalence of a given syndrome like fragile X but is done relatively late for there to be any clinical benefit. The latest studies, which were based on molecular testing, were closely tied to the offer of services to identified individuals and their families.

Table VI.3 shows the American experience. The two older studies (Nolin et al., 1991, 1992; Hagerman et al., 1988) involved mentally retarded children and adults, while the more recent ones (Hagerman et al., 1994b; Meadows et al., 1996; Crawford et al., 1999) targetted children with special educational needs. The project in New York State and the second project carried out in Colorado (Nolin et al., 1991, 1992; Hagerman et al., 1994b) involved prescreening using physical and/or behavioural criteria. Hagerman et al.'s study (1994b) was different in that it attempted to demonstrate the feasibility of prescreening performed by special education teachers, with subsequent referral to a specialized clinic. In this regard, this project may be seen as an improvement in the referral process and therefore an extension of the current clinical practice of diagnosis rather than a proactive diagnostic and screening strategy. However, the involvement of the research team in the schools seems important, the validity of the selection criteria still need to be validated (only one preliminary report has been published), and the gap between the feasibility of the pilot project and that of implementing a program inspired by it seems quite large.

Lastly, Table VI.4 summarizes a few of the features of the two most exhaustive diagnostic and screening programs reported to date. They are regional case-finding and cascade screening programs. The acceptability and impact of these two programs are the subject of a concurrent evaluation.

In Australia, a pilot project initiated in 1984 in the Sydney area became, in 1986, a diagnostic and screening program covering the entire state of New South Wales[229] (Turner et al., 1986). Initially, all individuals with mental retardation of unknown etiology receiving services in institutions, day centres or sheltered workshops were targetted, together with mentally retarded children in schools and special classes. When the program became regional in nature, prescreening based on a clinical examination was instituted in order to reduce the number of tests performed. In 1989, the program entered its maintenance phase, at which time it was children with mental retardation or developmental delay who were targetted, upon entering elementary school (Turner, 1992). In 1994, given the trend toward integrating mentally impaired children into regular schools, screening continued among mentally retarded boys entering high school. Since the tests were initially performed by cytogenetic analysis, the prevalence estimates had to be adjusted downward following retesting by means of molecular analysis (Turner et al., 1997) (see Section 1.2). Genetic counselling for the families and the offer of cascade screening were already part of the project. The acceptability of this approach and the impact of genetic counselling on reproductive decisions (see Section 6.2.1) have been the subject of an in-depth evaluation (Turner et al., 1997; Robinson et al., 1996).

In 1992, a regional diagnostic and screening program was instituted in southwestern Netherlands (de Vries et al., 1997, 1998d). Adults and children with mental retardation of unknown etiology who were in institutions or attending special schools were evaluated for prescreening purposes, with molecular testing subsequently proposed to those who were most at risk. The offer of genetic counselling was an integral part of program, but cascade screening is far less advanced there than in Australia, given that the program is much younger (see Section 6.2.1). The program's acceptability has also been studied by means of a survey among the participating and nonparticipating families.

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[229] The population of New South Wales is about 6.5 million.

Table VI.1: Experience with proactive diagnosis and screening in various European countries

Selection of Population	**Inclusion criteria**	**Method (n/N)**[1]	**Status**	**Objective(s)**
SWEDEN	***Gustafson et al., 1986***			
MR based on MR and death registers and on pediatric records.	Cohort: 1959-1970; etiology of MR unknown.	Cytogenetic analysis (12/89)	Research project	To determine the regional prevalence.
FINLAND	***Kähkönen et al., 1987***			
MR based on 1985 survey for screening for MR in regular schools.	11-15 years of age; etiology of MR unknown.	Cytogenetic analysis (6/111)	Research project	To determine the regional prevalence.
SPAIN	***Gabarron et al., 1992***			
MR in students in special schools and adults in sheltered workshops.	♂, 1-40 years of age; etiology of MR unknown.	Cytogenetic analysis (22/223)	Research project + clinical follow-up	To determine the regional prevalence and provide genetic counselling to families.
DENMARK	***Tranebjaerg et al., 1994***			
Learning disabilities, according to a register of students in special and regular schools.	8-10 years of age.	Cytogenetic analysis (0/102)	Research project	To determine the regional prevalence.

MR: mental retardation

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[1] n/N = number of individuals identified (FMs + PMs, if molecular testing) over the number of individuals tested. Depending on the exclusion criteria, the number of individuals identified may or may not include persons already known to be affected. These figures are therefore not necessarily those used to calculate the syndrome's prevalence.

Table VI.2: Experience with proactive diagnosis and screening in various parts of Great Britain

Selection of Population	Inclusion criteria	Method (n/N)[1]	Status	Objective(s)
COVENTRY	***Webb et al., 1986; Morton et al., 1997***			
Children in special schools + hospitals and residential facilities for the mentally retarded.	4-16 years of age; etiology of MR unknown.	1. Cytogenetic analysis (29/347) 2. Retesting with molecular analysis (7/24).	Research project	To determine the regional prevalence.
WESSEX	***Jacobs et al., 1993; Murray et al., 1996; Youings et al., 2000***			
Students with special educational needs.	5-18 years of age; etiology of MR unknown.	Molecular testing (4+0/254)	Pilot project + clinical follow-up	To evaluate the test and assesses the program's feasibility; research; genetic counselling for families.
Students with learning disabilities in regular schools.	Boys: 5-18 years of age; etiology of MR unknown.	Molecular testing (5+1/1,013)	Screening program	Research and clinical service; to determine the regional prevalence.
Students with special educational needs.	Boys: 5-18 years of age; etiology of MR unknown.	Molecular testing (20+2/3,738)	Screening program	Research and clinical service; to determine the regional prevalence.
OXFORDSHIRE	***Slaney et al., 1995***			
Children with learning disabilities in special schools.	3-18 years of age; etiology of MR unknown.	Molecular testing (4/154)	Research project + clinical follow-up	To determine the regional prevalence and provide genetic counselling to families.

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[1] n/N = number of individuals identified (FMs + PMs, if molecular testing) over the number of individuals tested. Depending on the exclusion criteria, the number of individuals identified may or may not include persons already known to be affected. These figures are therefore not necessarily those used to calculate the syndrome's prevalence.

Table VI.3: Experience with proactive diagnosis and screening in various parts of the United States

Selection of Population	**Inclusion criteria**	**Method (n/N)[1]**	**Status**	**Objective(s)**
NEW YORK STATE	***Nolin et al., 1991, 1992***			
Mentally retarded ♂ in institutions or residential facilities.	13-81 years of age; etiology of MR unknown; prescreening by means of a physical examination; ♀ relatives.	Cytogenetic analysis (43/489)	Regional program	Case-finding and providing genetic counselling to families
COLORADO	***Hagerman et al., 1988, 1994b***			
Institutionalized mentally retarded individuals.	4-69 years of age; etiology of MR unknown.	Cytogenetic (11/440)	Research project	To determine the frequency in institutions.
Children with special educational needs in regular schools.	2-18 years of age; prescreening by special education teachers.	Molecular testing (4+1/439)	Pilot project + clinical follow-up	To evaluate tools, assess the program's feasibility and provide genetic counselling to families.
GEORGIA	***Meadows et al., 1996; Crawford et al., 1999***			
Children with special educational needs in special classes	7-10 years of age.	Molecular testing (2+4/1,279)	Research project	To determine the frequency among children with special educational needs.
Children with special educational needs in special classes	7-10 years of age.	Molecular testing (5+2/2,873)	Research project	To determine the regional prevalence.

[1] n/N = number of individuals identified (FMs + PMs, if molecular testing) over the number of individuals tested. Depending on the exclusion criteria, the number of individuals identified may or may not include persons already known to be affected. These figures are therefore not necessarily those used to calculate the syndrome's prevalence.

Table VI.4: Experience with proactive diagnosis and screening in Australia and the Netherlands

Selection of Population	Inclusion criteria	Method (n/N)[1]	Status	Purpose(s)
AUSTRALIA	***Turner et al., 1986, 1992, [illegible]7***			
MR in adults in institutions, workshops or day centres; children in special schools or classes.	Etiology of MR unknown.	Cytogenetic analysis (40/921)	Pilot project	To determine the regional prevalence, case-finding and providing genetic counselling to families.
MR in adults in institutions, workshops or day centres; children in special schools or classes; + MR upon entering the elementary level (special schools or classes)	Etiology of MR unknown; prescreening by physical examination.	Cytogenetic analysis (253/3,862)	Regional program	Case-finding and providing genetic counselling to families; and to assess the program's acceptability and the impact on reproductive decisions.
MR upon entering high school (regular schools).	Etiology of MR unknown; prescreening by means of a clinical examination.	Molecular testing (245/?)	Regional program	To check the prevalence; case-finding and providing genetic counselling to families; and to assess the program's acceptability and the impact on reproductive decisions.
HOLLAND	***de Vries, 1997 et al., 1998d***			
MR in institutions and special schools.	4-89 years of age; etiology of MR unknown; prescreening by clinical examination.	Molecular testing (11+0 /1,531)	Regional program	To determine the regional prevalence, assess the program's feasibility and acceptability, and provide genetic counselling to families.

[1] n/N = number of individuals identified (FMs + PMs, if molecular testing) over the number of individuals tested. Depending on the exclusion criteria, the number of individuals identified may or may not include persons already known to be affected. These figures are therefore not necessarily those used to calculate the syndrome's prevalence.

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